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TITLE: Preparation of Chemicals and Bulk Drug Substances for the
U.S. Army Drug Development Program

PRINCIPAL INVESTIGATOR: Jaroslav F. Novotny, Ph.D.

CONTRACTING ORGANIZATION: Starks Associates, Inc.
Buffalo, NY 14213

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U. S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

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The following target compounds have been synthesized during this period: 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; 1,9(2*H*,10*H*)-acridinedione, 7-chloro-3-(2,4-dichlorophenyl)-3,4-dihydro-; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine; product(s) obtained by reacting EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2*H*)-acridinone; product(s) obtained on condensation of 6-aminopiperonal with 2,5-dihydroxy-7-methyl-6-cyanoindolizine; (*S*)-15-chloro-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo[4,5-*g*]pyrano[3',4': 6,7]indolizino-[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione Syn.: 9-chloro-10,11-(methylenedioxy)-20(*S*)-camptothecin; (*S*)-7-Ethyl-7-hydroxy-10*H*-2,2-difluoro-1,3-dioxolo[4,5-*g*]pyrano[3',4': 6,7]-indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione Syn.: 10,11-(difluoromethylenedioxy)-20(*S*)-camptothecin; butyric acid, 4-(4-chlorophenyl)-4(*R*)-[10(β)-dihydroartemisininoxy]-; artemisinin; dihydroartemisinin; artelinic acid, methyl ester; artelinic acid.

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I SUMMARY

A broad spectrum of chemical compounds for U. S. Army Drug Development Program were synthesized during this period. Seventeen candidate drugs were delivered. New synthetic methods were designed for four products transmitted to WRAIR.

The purity of all target compounds and intermediates was rigorously checked by a series of physical and chemical tests. Methods have been developed for compounds not previously recorded in the chemical literature. The cost for raw materials and labor were kept under strict control by minimizing the turn-around-time for each requested material.

The following target compounds have been synthesized during this period: 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; 1,9(2*H*,10*H*)-acridinedione, 7-chloro-3-(2,4-dichlorophenyl)-3,4-dihydro-; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine; product(s) obtained by reacting EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2*H*)-acridinone; product(s) obtained on condensation of 6-aminopiperonal with 2,5-dihydroxy-7-methyl-6-cyanoindolizine; (S)-15-chloro-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo[4,5-*g*]pyrano[3',4': 6,7]indolizino-[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione Syn.: 9-chloro-10,11-(methylenedioxy)-20(S)-camptothecin; (S)-7-Ethyl-7-hydroxy-10*H*-2,2-difluoro-1,3-dioxolo[4,5-*g*]pyrano[3',4': 6,7]-indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione Syn.: 10,11-(difluoromethylenedioxy)-20(S)-camptothecin; butyric acid, 4-(4-chlorophenyl)-4(R)-[10(β)-dihydroartemisininoxy]-; artemisinin; dihydroartemisinin; artelinic acid, methyl ester; artelinic acid.

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____In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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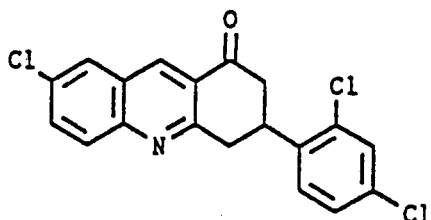
____In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Jaroslav F. Novotny 23/12/97
Jaroslav F. Novotny, PI / Date

III. CUMULATIVE LIST OF REQUESTED TARGET COMPOUNDS DELIVERED TO
WALTER REED ARMY INSTITUTE OF RESEARCH FROM DECEMBER 1, 1996
TO NOVEMBER 30, 1997

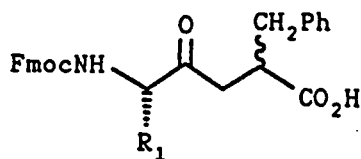
Compound

Cumulative No.*



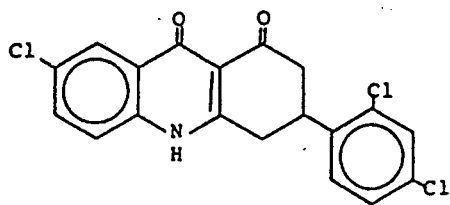
1. 1(2H)-Acridinone,
7-chloro-3,4-dihydro-
3-(2,4-dichlorophenyl)-

1230



2. Fmoc-L-Gln(Trt)Ψ(COCH₂)-
D,L-Phe

1231



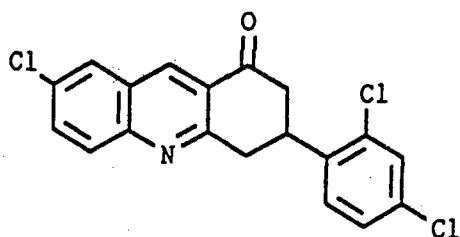
3. 1,9(2H,10H)-Acridinedione,
7-chloro-3-(2,4-dichlorophenyl)-
3,4-dihydro-

1232

*Additional information may be found in the Cumulative List
on page 7, this report.

Compound

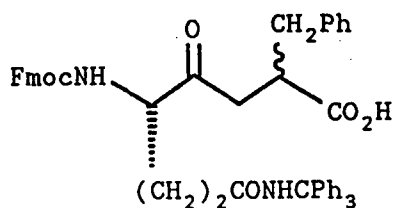
Cumulative No.



4. 1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- 1233

5. Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine 1234

6. Product(s) obtained by reacting EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2H)-acridinone 1235

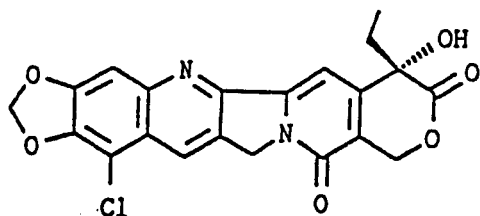


7. Fmoc-L-Gln(Trt)Ψ(COCH2)-D,L-Phe 1236

8. Product(s) obtained on condensation of 6-aminopiperonal with 2,5-dihydroxy-7-methyl-6-cyanoindolizine 1237

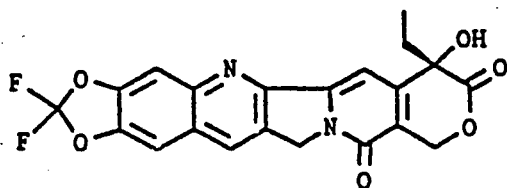
Compound

Cumulative No.



9. (S)-15-Chloro-7-ethyl-7-hydroxy-10H-1,3-dioxolo-
[4,5-g]pyrano[3',4' : 6,7]-
indolizino[1,2-b]quinoline-
8,11-(7H,13H)-dione
Syn.: 9-Chloro-10,11-(methylene-
dioxy)-20(S)-camptothecin

1238

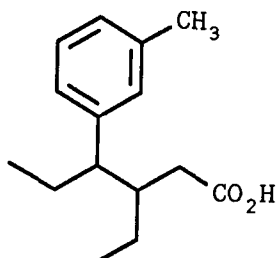


10. (S)-7-Ethyl-7-hydroxy-10H-
2,2-difluoro-1,3-dioxolo[4,5-g]-
pyrano[3',4' : 6,7]indolizino[1,2-b]-
quinoline-8,11-(7H,13H)-dione
Syn.: 10,11-(Difluoromethylenedioxy)-
20(S)-camptothecin

1239

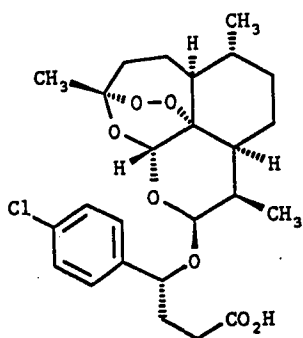
Compound

Cumulative No.



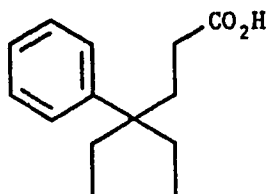
11. Hexanoic acid, 3-ethyl-4-(3-methylphenyl)-

1240



12. Butyric acid, 4-(4-chlorophenyl)-4(R)-[10(β)-dihydroartemisinin-10-yl]-

1241

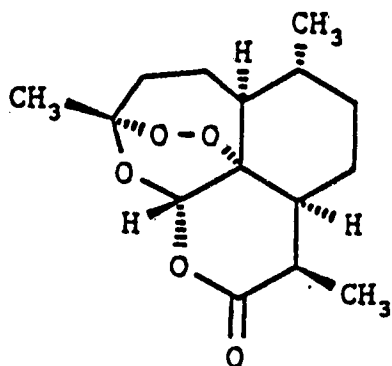


13. Hexanoic acid, 3-ethyl-4-(3-methylphenyl)-

1242

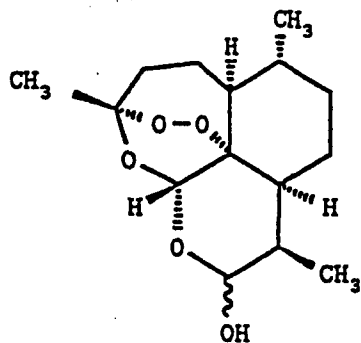
Compound

Cumulative No.



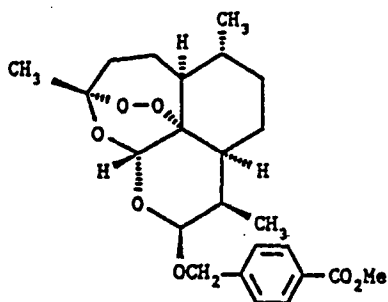
14. Artemisinin

1243



15. Dihydroartemisinin

1244

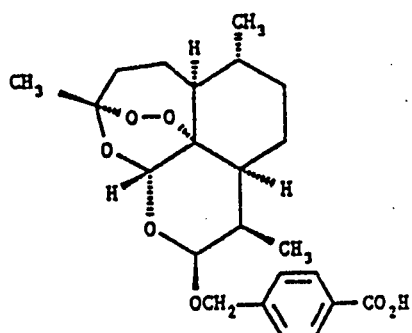


16. Artelinic acid, methyl ester

1245

Compound

Cumulative No.



17. Artelinic acid

1246

IV. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO
WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM
DECEMBER 1, 1996 TO NOVEMBER 30, 1997

The previous Cumulative List covering the period from July 1, 1965 to June 30, 1973 may be found in Starks Associates, Inc. Final Comprehensive Report dated June 30, 1973 pages 54-97, Contract No. DA-49-193-MD-2751. The list covering the period from July 1, 1973 to September 28, 1979 may be found in Starks Associates, Inc. Final Comprehensive Report dated September 1979, pages 82-241, Contract No. DAMD17-73-C-3159. The list covering the period from September 29, 1979 to September 14, 1983 may be found in Starks Associates, Inc. Final Comprehensive Report dated September 1983, pages 56-163, Contract No. DAMD17-79-C-9170. The list covering the period from September 15, 1983 to March 14, 1989 may be found in Starks Associates, Inc. Final Comprehensive Report dated March 14, 1989, pages 55-116, Contract No. DAMD17-83-C-3206. The list covering the period from March 15, 1989 to November 30, 1992 may be found in Starks Associates, Inc. Final Comprehensive Report dated November 30, 1992, pages 35-58, Contract No. DAMD17-89-C-9058. The list covering the period from December 1, 1992 to November 30, 1993 may be found in Starks Associates, Inc., Annual Report dated November 30, 1993 pages 4-5, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1993 to November 30, 1994 may be found in Starks Associates, Inc., Annual Report dated November 30, 1994 pages 4-5, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1994 to November 30, 1995 may be found in Starks Associates, Inc. Annual Report dated January 1996 pages 10-16, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1995 to November 30, 1996 may be found in Starks Associates, Inc. Annual Report dated December 1996 pages 7-9, Contract No. DAMD17-93-C-3003.

| <u>Cumu- lative No.</u> | <u>Compound</u> | <u>Amount</u> | <u>BN#</u> | <u>WR#</u> | <u>Starks Assoc. Report</u> |
|---------------------------------|--|---------------|------------|------------|-------------------------------------|
| 1230 | 1(2H)-Acridinone, 7-chloro-3,4- dihydro-3-(2,4- dichlorophenyl)- | 2.1 g | BN83315 | 280850 | 127 |
| 1231 | Fmoc-L-Gln(Trt)ψ- (COCH ₂)-D,L-Phe | 188 mg | BN84134 | 280905 | 127 |
| 1232 | 1,9(2H,10H)-Acridine- dione, 7-chloro-3- (2,4-dichlorophenyl)- 3,4-dihydro- | 2.1 g | BN85284 | 243246 | 127 |

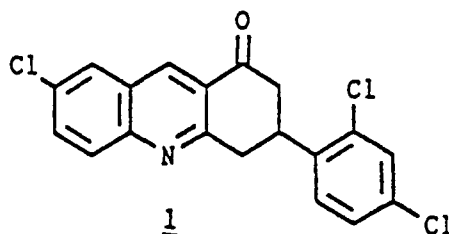
| <u>Cumu- lative No.</u> | <u>Compound</u> | <u>Amount</u> | <u>BN#</u> | <u>WR#</u> | <u>Starks Assoc. Report</u> |
|---------------------------------|--|---------------|------------|------------|-------------------------------------|
| 1233 | 1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- | 5.0 g | BN85293 | 280850 | 127 |
| 1234 | Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine | 200 mg | BN85471 | 280993 | 127 |
| 1235 | Product(s) obtained by reacting EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2H)-acridinone | 15 mg | BN85480 | 280994 | 127 |
| 1236 | Fmoc-L-Gln(Trt)Ψ(COCH ₂)-D,L-Phe | 630 mg | BN87644 | 281074 | 128 |
| 1237 | Product(s) obtained on condensation of 6-amino-piperonal with 2,5-dihydroxy-7-methyl-6-cyanoindolizine | 100 mg | | | 128 |
| 1238 | (S)-15-Chloro-7-ethyl-7-hydroxy-10H-1,3-dioxolo-[4,5-g]pyrano[3',4':6,7]-indolizino[1,2-b]-quinoline-8,11-(7H,13H)-dione Syn.: 9-chloro-10,11-(methylenedioxy)-20-(S)camptothecin | 3.9 g | BN89353 | 279773 | 129 |
| 1239 | (S)-7-Ethyl-7-hydroxy-10H-2,2-difluoro-1,3-dioxolo[4,5-g]pyrano-[3',4' : 6,7]indolizino-[1,2-b]quinoline-8,11-(7H,13H)-dione Syn.: 10,11-(Difluoro-methylenedioxy)-20(S)-camptothecin | 700 mg | BN89684 | 281187 | 129 |
| 1240 | Hexanoic acid, 3-ethyl-4-(3-methylphenyl)- | 25 mg | BN89693 | 281181 | 129 |

| <u>Cumu- lative No.</u> | <u>Compound</u> | <u>Amount</u> | <u>BN#</u> | <u>WR#</u> | <u>Starks Assoc. Report</u> |
|---------------------------------|---|---------------|------------|------------|-------------------------------------|
| 1241 | Butyric acid, 4-(4-chlorophenyl)-4(R)-[10(β)-dihydro-artemisininoxy]- | 25.2 g | BN92092 | 280325 | 129 |
| 1242 | Hexanoic acid, 4-ethyl-4-phenyl- | 99 mg | BN92743 | 281380 | 130 |
| 1243 | Artemisinin | 10.0 g | BN92752 | 249309 | 130 |
| 1244 | Dihydroartemisinin | 10.0 g | BN95075 | 253997 | 130 |
| 1245 | Artelinic acid, methyl ester | 12.0 g | BN95066 | 255608 | 130 |
| 1246 | Artelinic acid | 10.0 g | BN95084 | 255663 | 130 |

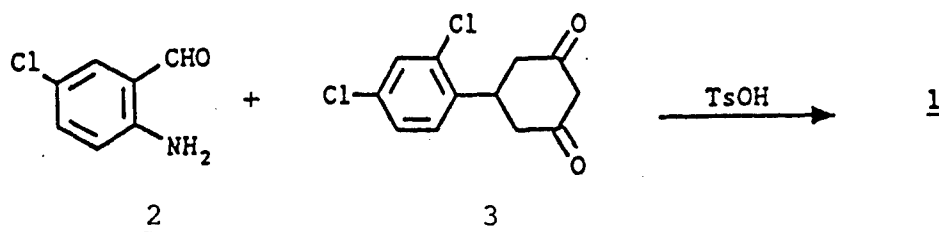
V. DISCUSSION OF RESEARCH AND TARGET COMPOUNDS

1. 1(2H)Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (1)

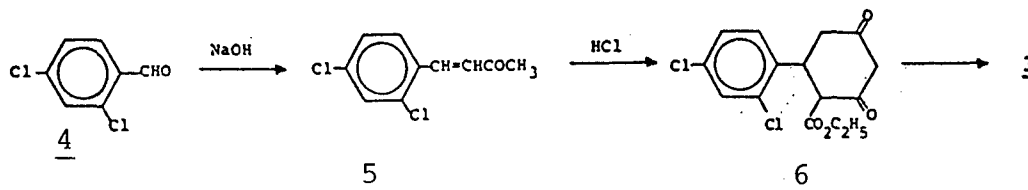
The target compound 1 was prepared by



reacting 5-chloro-2-aminobenzaldehyde (2) with 5-(2,4-dichlorophenyl)cyclohexane-1,3-dione (3) in the presence of *p*-toluenesulfonic acid.



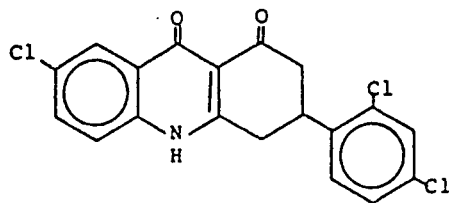
Compound 2 was obtained by reducing the corresponding nitro derivative with ferrous sulfate, heptahydrate. Material 3 was prepared by a three step synthesis shown below.¹ Dichlorobenzaldehyde 4 was reacted



with acetone in the presence of base to give 5 in 67% yield. Reaction of 5 with diethyl malonate in the presence of sodium ethoxide gave 6 in 72% yield. Ester 6 was hydrolyzed and decarboxylated to give 3 in 79% yield.

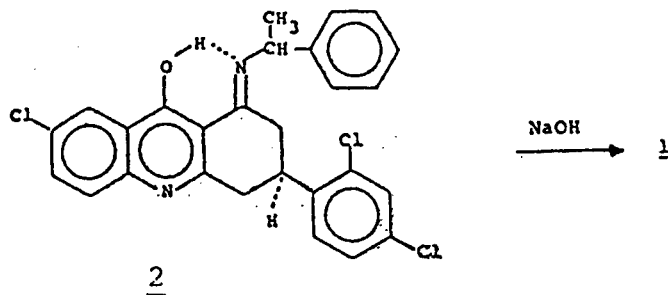
2. 1,9(2H,10H)-Acridinedione, 7-chloro-3-(2,4-dichlorophenyl)-3,4-dihydro- (1)

The target compound 1 was prepared



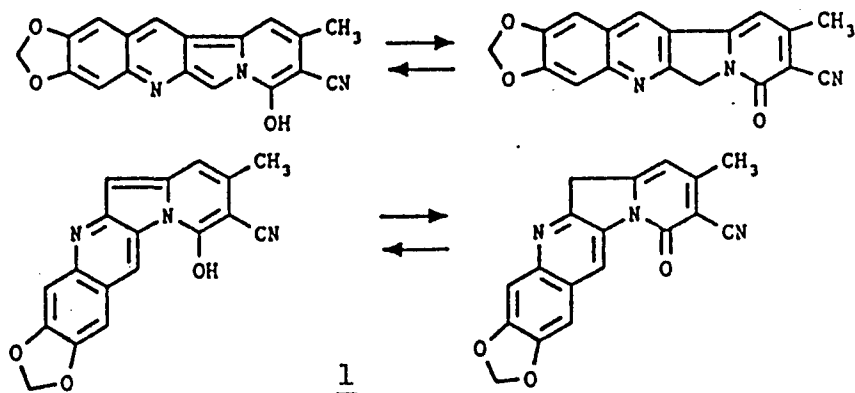
1

by hydrolyzing 2 with NaOH. Material 2 was



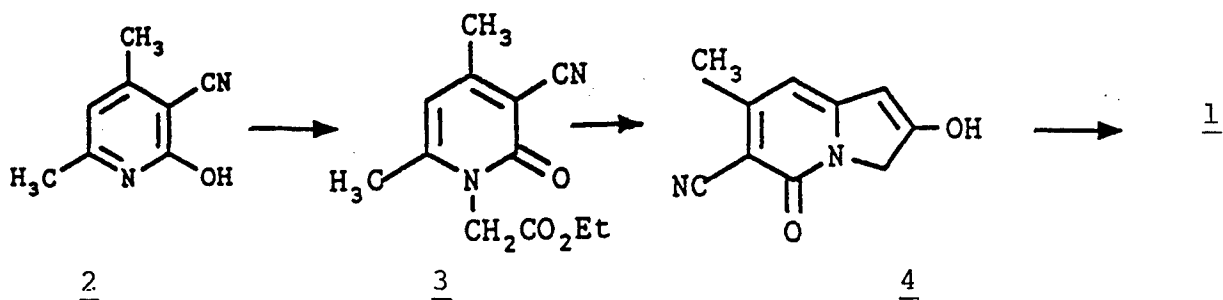
obtained from stock and was previously prepared from 1 and *l*-(-)-1-methylbenzylamine.²

3. Product obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine (1).



Possible structure for 1

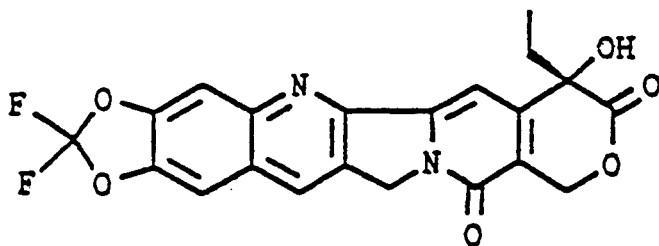
The above material was prepared by the following sequence of reactions. Commercially



available 2 was reacted with ethyl bromoacetate in the presence of a base to give 3 in 39% yield. The action of sodium hydroxide on 3 gave 4 in 87% yield. Material 4 was condensed with 6-aminopiperonal in the presence of *p*-toluene-sulfonic acid to give 1. A portion of this material was transmitted to WRAIR, although the product was not fully characterized.

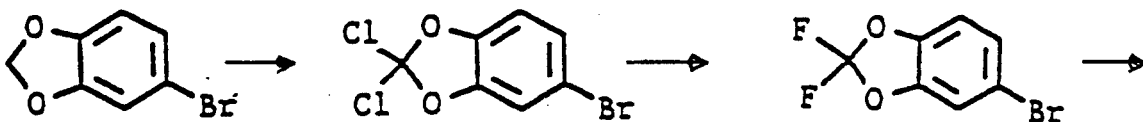
4. (S)-7-Ethyl-7-hydroxy-10H-2,2-difluoro-1,3-dioxolo[4,5-g]-pyrano[3',4' : 6,7]indolizino[1,2-b]quinoline-8,11-(7H,13H)-dione (1)

The target compound 1 was prepared by the

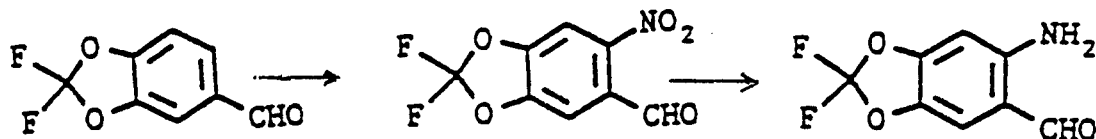


1

following sequence of reactions. Commercially available 3,4-(methylenedioxy)bromobenzene (2) was reacted with phosphorus pentachloride to give the dichloro derivative in 87% yield. Compound 3 gave with antimony trifluoride the difluoro compound 4 in 85% yield.

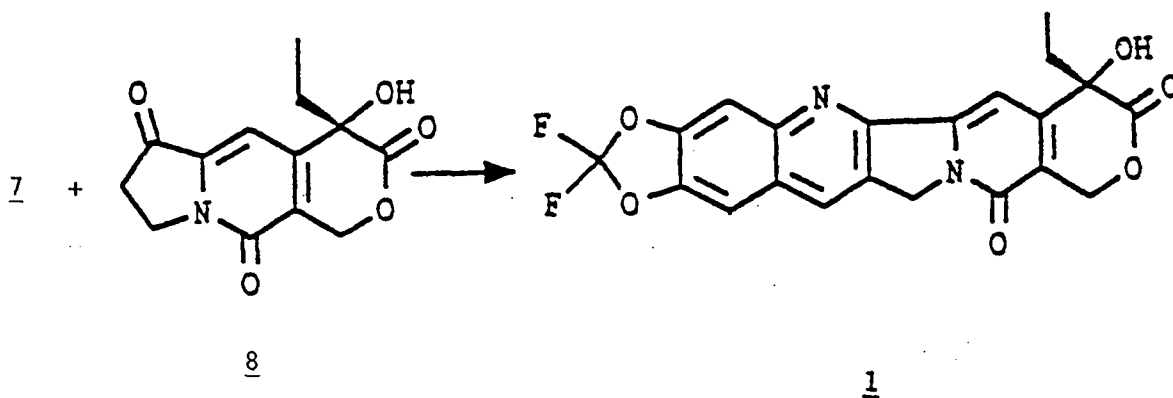


4



7

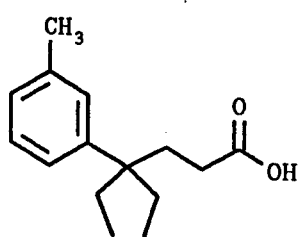
The reaction of 4 with butyllithium and DMF yielded the aldehyde 5 in 59% yield. Preparation of 3,4 & 5 has been described in the literature.³ Aldehyde 5 was nitrated with 90% nitric acid and trifluoromethanesulfonic acid to give the unknown material 6 in 72% yield. The reaction of 6 with ferrous sulfate gave the amino compound 7 in 33% yield. Material 7 was condensed with



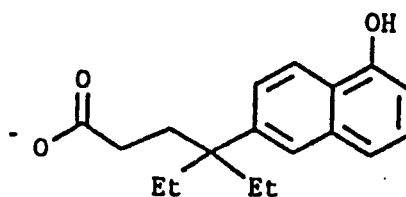
trione 8 to give the target material 1 in 37% yield. Materials 6,7 & 1 are unknown to the chemical literature.

5. Hexanoic acid, 3-ethyl-4-(3-methylphenyl)- (10b)

The material was isolated during an attempted synthesis of model compound 1. This preparation was a model for the synthesis of ligand 09E.

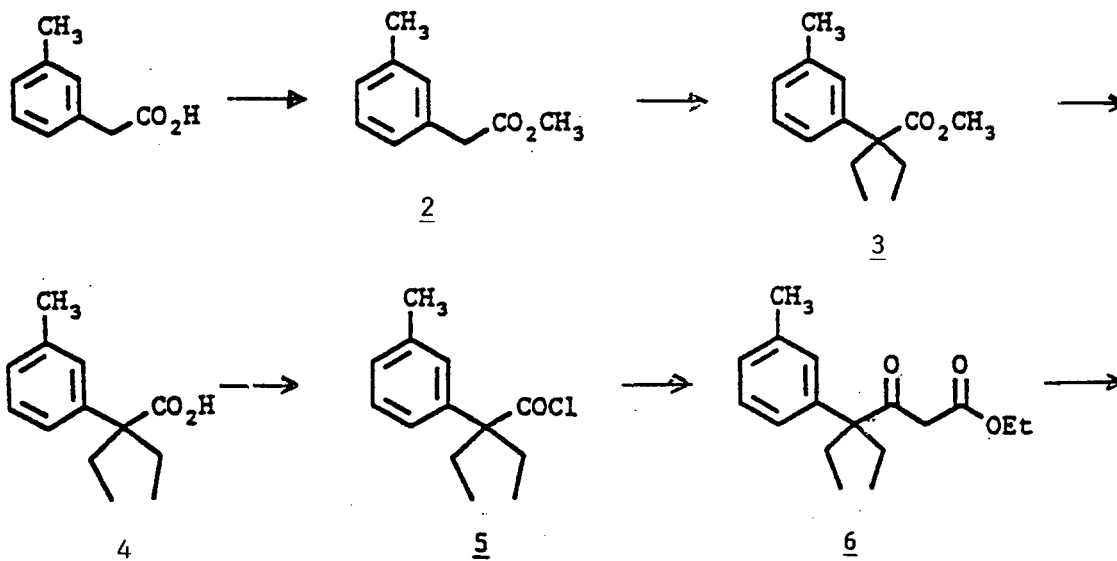


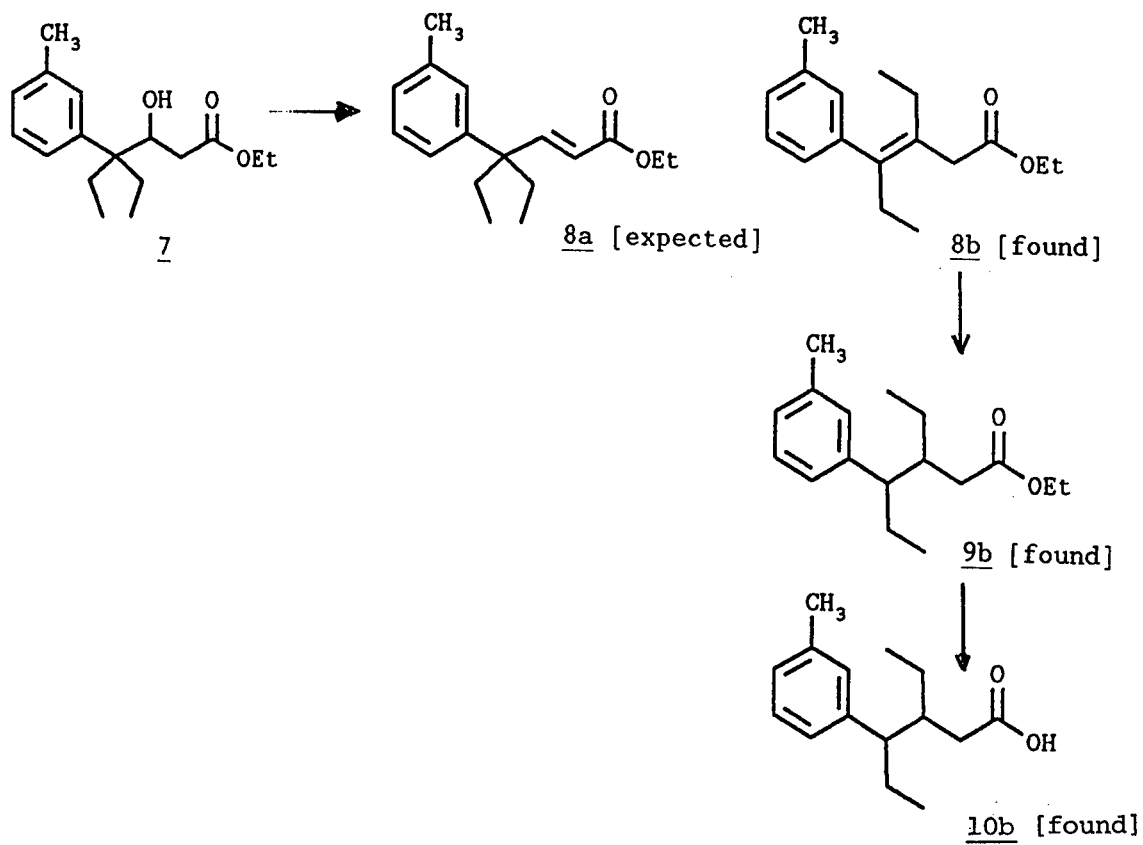
1



Ligand 09E

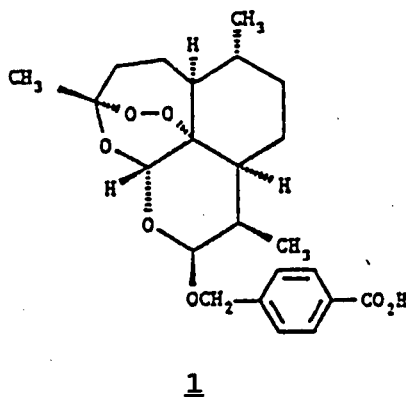
m-Tolylacetic acid was esterified to give the ester 2 in 99% yield. Material 2 was reacted with ethyl iodide to give 3 in 81% yield. The ester 3 was hydrolyzed to afford the acid 4 in 92% yield. The acid was converted to the acid chloride 5 then reacted with monoethyl malonate to give 6 in 40% yield. The ketoester 6 was reduced with sodium borohydride to give the hydroxy compound 7. Dehydration of 7 with *p*-toluenesulfonic acid did not yield the expected 8a but rather the material 8b. Reduction of 8b to 9b followed by hydrolysis gave the material 10b.



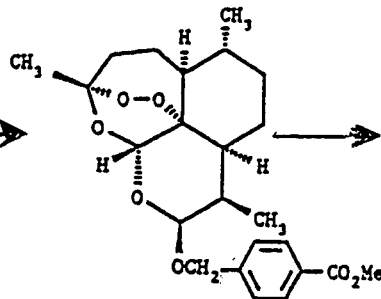
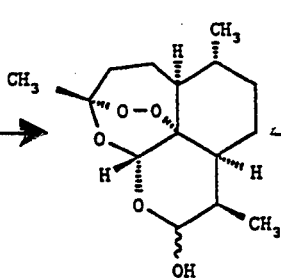
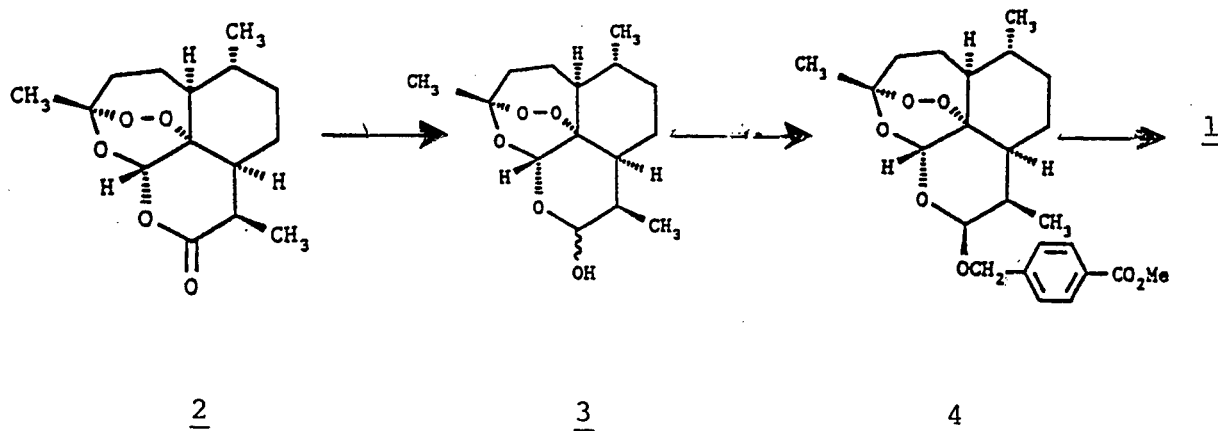


6. Artelinic acid (1)

The target compound 1 was prepared by the following sequence of reactions. Commercially available artemisinin



(2) was reduced with sodium borohydride to give a mixture of dihydroartemisinines (3) in 84% yield. This was not purified but immediately reacted with methyl 4-hydroxymethylbenzoate in the presence of boron trifluoride diethyl etherate to give the methyl ester of artelinic acid (4) in 67% yield. Hydrolysis of the ester with methanolic KOH at RT gave the target compound 1 in 86% yield.



1

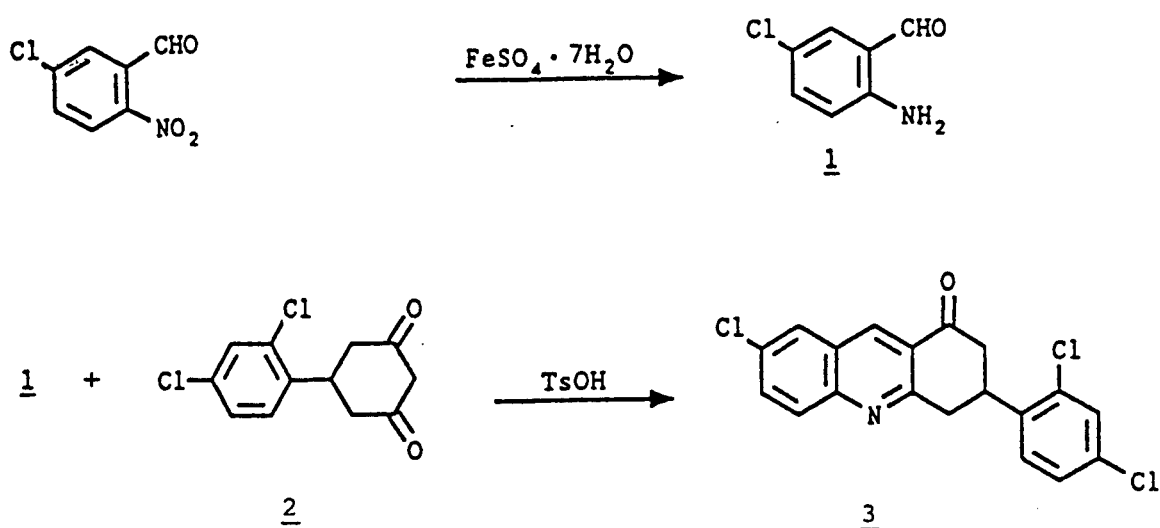
VI. RESEARCH AND KNOWN TARGET COMPOUNDS AND INTERMEDIATES
COMPLETED AND DELIVERED TO WALTER REED ARMY INSTITUTE
OF RESEARCH FROM DECEMBER 1, 1996 TO NOVEMBER 30, 1997

A. Infectious Disease Related Compounds and Intermediates

1. 1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

The target compound (3) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

5-Chloro-2-aminobenzaldehyde (1)

To a boiling solution of ferrous sulfate, heptahydrate (53.0 g, 0.19 mol) in H_2O (250 mL) was added a solution of 5-chloro-2-nitrobenzaldehyde (5.0 g, 26.9 mmol) in 50% aqueous ethanol (250 mL). The solution was boiled for one minute and then concentrated ammonium hydroxide (70 mL) was added in

10 mL portions. After the addition the suspension was boiled for 10 min, filtered hot, and the solid was washed with boiling H₂O (2 x 100 mL). The filtrate and washings were combined, cooled, and the solid that separated was collected. This was dissolved in CH₂Cl₂ (100 mL), washed with H₂O (50 mL), dried over Na₂SO₄ and after removal of solvent in vacuo 2.2 g of 1 was obtained, which was suitable for the next step.

5-(2,4-Dichlorophenyl)cyclohexane-1,3-dione (2)

Please refer to Starks Associates, Inc. Annual Summary Report dated June 1987 (July 1, 1986 to June 30, 1987) Contract DAMD17-83-C-3206, pg. 28.

1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

To a mixture of 5-chloro-2-aminobenzaldehyde (1) (3.2 g, 20.6 mmol) and 5-(2,4-dichlorophenyl)cyclohexane-1,3-dione (2) (5.0 g, 19.5 mmol) in toluene (1 L) was added *p*-toluene-sulfonic acid monohydrate (300 mg) and the mixture was heated at slow reflux using Dean Stark apparatus to remove water. After 2 h of reflux the reaction was cooled and concentrated in vacuo. The residue was triturated with hot CH₃OH (100 mL). The solid was collected then crystallized from glacial acetic acid (150 mL); yield 2.3 g (32.0%) mp 222-223°C. A portion (2.1 g) was transmitted to WRAIR on December 4, 1996 (NJ24-67-3).

Anal.

| | | <u>C</u> | <u>H</u> | <u>N</u> |
|------------|--|----------|----------|----------|
| Calc'd for | C ₁₉ H ₁₂ Cl ₃ NO | 60.59 | 3.21 | 3.72 |
| | Found | 60.62 | 3.35 | 3.73 |

Spectral Data

Infrared (KBr)

Major bands: 3430, 3060, 1690, 1610, 1590, 1555,
1475, 1450, 1430, 1395, 1370, 1300,
1270, 1230, 1205, 1190, 1130, 1100,
1070, 1050, 1015, 930 cm^{-1} .

Ultraviolet (CHCl_3)

λ_{max} 260 nm ($\log \epsilon$ 4.75); 325 nm (3.93).

Nuclear Magnetic Resonance (TFA-d)

δ 9.70 (s, 1, H-9); 8.45 (s, 1, H-8); 8.32 (m, 2,
H-5 and H-6); 7.53 (d, 1, C-3'), 7.37 (m, 1, C-5');
7.34 (d, 1, C-6'); 4.36 (m, 1, C-3); 3.97 (m, 2,
C-4); 3.39 (m, 2, C-2).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; visulization - UV.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----------------------|-----------------|----------------|
| Hexane-acetone (4:1) | 0.36 | Single spot |
| Hexane-EtOAc (2:1) | 0.74 | Single spot |

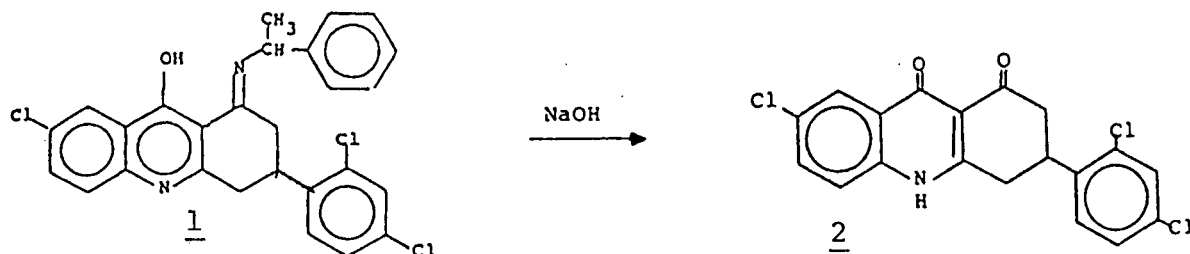
Source of Materials

- | | | |
|----|--|----------------------------|
| 1. | 5-Chloro-2-nitrobenzaldehyde | Aldrich Chemical Co., Inc. |
| 2. | $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ | Sigma |
| 3. | CH_2Cl_2 | J.T. Baker Chemical Co. |
| 4. | Na_2SO_4 | J.T. Baker Chemical Co. |
| 5. | 5-(2,4-Dichlorophenyl)- cyclohexane-1,3-dione | Starks Associates, Inc. |
| 6. | Toluene | J.T. Baker Chemical Co. |
| 7. | <i>p</i> -Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 8. | Methanol | J.T. Baker Chemical Co. |
| 9. | Acetic acid | J.T. Baker Chemical Co. |

2. 1,9(2H,10H)-Acridinedione, 7-chloro-3-(2,4-dichloro-phenyl)-3,4-dihydro- (2)

The target compound 2 was prepared by the following sequence of reactions:

Reactions Sequence



Experimental

(R)-7-Chloro-3-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-[(1-(S)-phenylethyl)imino]-9-acridinol (1)

Please refer to Starks Associates Inc. Annual Summary Report dated June 1987, p. 37, Contract DAMD17-83-C-3206.

1,9(2H,10H)-Acridinedione, 7-chloro-3-(2,4-dichloro-phenyl)-3,4-dihydro (2)

A solution of 1 (20.0 g, 40.3 mmol) and 50% NaOH (60 mL) in THF (400 mL) was heated at 60°C for 2 h, cooled then poured into H₂O (3 L). The solid that separated was collected on a filter then suspended in CH₃OH (1 L), and the suspension was acidified then refluxed for 15 min. The solid was collected, then dried, yield 9.8 g (61.9%). A portion (5.8 g) was suspended in AcOH (200 mL) refluxed for 10 min, cooled, suspended in 10% Na₂CO₃ solution (100 mL), filtered then washed until the filtrate was neutral then dried. Yield 5.0 g (86.2% recovery), mp >300°C.⁴ A portion (2.1 g) was transmitted to WRAIR on January 15, 1997 (Lot No. NJ24-85-1).

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> |
|-----------------------------------|----------|----------|----------|
| Calc'd for $C_{19}H_{12}Cl_3NO_2$ | 58.12 | 3.08 | 3.57 |
| Found | 58.17 | 3.10 | 3.56 |

Spectral Data

Infrared (KBr)

Major bands: 3400, 3060, 2980, 2950, 1660, 1625, 1500, 1560, 1510, 1470, 1400, 1340, 1310, 1270, 1100, 1050, 1020 cm^{-1} .

Ultraviolet (2-methoxyethanol)

λ_{max} 257 nm (log ϵ 4.19); 266 nm (4.24); 280 nm (3.91); 307 nm (4.12); 316 nm (4.10); 335 nm (3.79); 360 nm (3.23).

Nuclear Magnetic Resonance (TFA-d)

δ 8.59 (s, 1, H-3'); 8.13 (d, 1, J=9.0 Hz, H-5'); 8.02 (d, 1, J=9.0 Hz, H-6'); 7.50 (s, 1 H-8); 7.34 (d, 1, J= 8.4 Hz, H-5); 7.29 (d, 1, J= 8.4 Hz, H-6); 4.27 (p, J= 12.9, 3.3, 2.9, 11.9 Hz, H-3); 3.73 (m, 2, H's at C-2); 3.29 (d, 2, H's at C-4).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; visualization - UV.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----------------------------------|-----------------|----------------|
| 1. MeOH-NH ₄ OH (9:1) | 0.84 | Homogeneous |
| 2. Acetone-hexane (1:1) | 0.41 | Homogeneous |

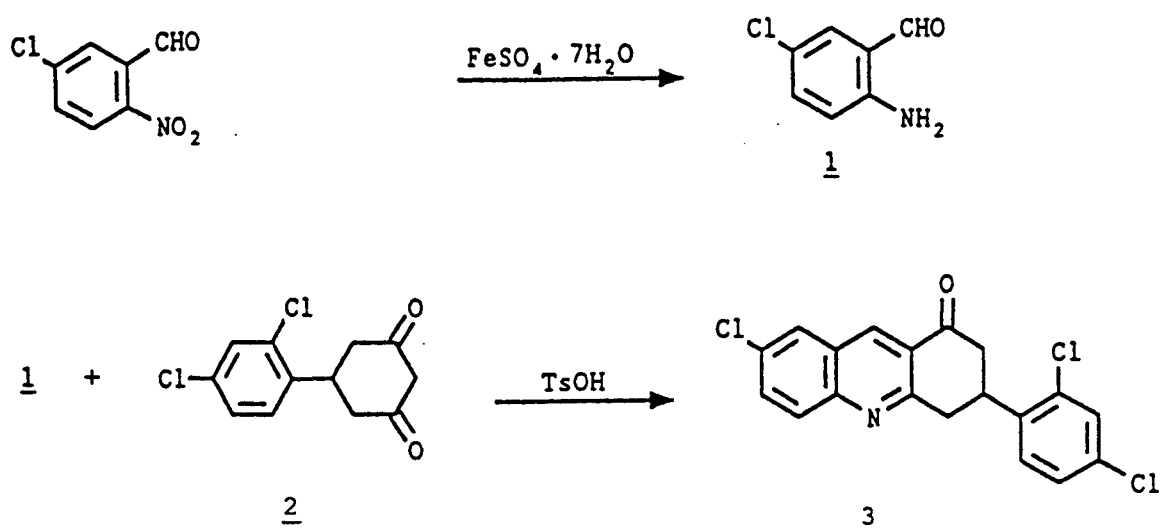
Source of Material

- | | | |
|----|--|----------------------------|
| 1. | (R)-7-Chloro-3-(2,4-dichloro-phenyl)-1,2,3,4-tetrahydro-1-[(1-(S)-phenylethyl)imino]-9-acridinol | Starks Associates, Inc. |
| 2. | THF | Aldrich Chemical Co., Inc. |
| 3. | NaOH | Aldrich Chemical Co., Inc. |
| 4. | CH ₃ OH | J.T. Baker Chemical Co. |
| 5. | AcOH | J.T. Baker Chemical Co. |
| 6. | Na ₂ CO ₃ | Aldrich Chemical Co., Inc. |

3. 1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

The target compound (3) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

5-Chloro-2-aminobenzaldehyde (1)

To a boiling solution of ferrous sulfate, heptahydrate (53.0 g, 0.19 mol) in H_2O (250 mL) was added a solution of 5-chloro-2-nitrobenzaldehyde (5.0 g, 26.9 mmol) in 50% aqueous ethanol (250 mL). The solution was boiled for one minute and then concentrated ammonium hydroxide (70 mL) was added in 10 mL portions. After the addition the suspension was boiled for 10 min, filtered hot, and the solid was washed with boiling H_2O (2 x 100 mL). The filtrate and washings were combined, cooled, and the solid that separated was collected. This was dissolved in CH_2Cl_2 (100 mL), washed with H_2O (50 mL),

dried over Na_2SO_4 and after removal of solvent in vacuo 2.0 g of 1 was obtained, which was suitable for the next step.

5-(2,4-Dichlorophenyl)cyclohexane-1,3-dione (2)

Please refer to Starks Associates, Inc. Annual Summary Report dated June 1987 (July 1, 1986 to June 30, 1987) Contract DAMD17-83-C-3206, pg. 27.

1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

To a mixture of 5-chloro-2-aminobenzaldehyde (1) (2.0 g, 12.8 mmol) and 5-(2,4-dichlorophenyl)cyclohexane-1,3-dione (2) (3.2 g, 12.5 mmol) in toluene (1 L) was added *p*-toluene-sulfonic acid monohydrate (300 mg) and the mixture was heated at slow reflux using Dean Stark apparatus to remove water. After 2 h of reflux the reaction was cooled and concentrated in vacuo. The residue was triturated with hot CH_3OH (100 mL). The solid was collected, combined with 4.9 g of similar material obtained in other reactions then crystallized from glacial acetic acid (100 mL); yield 6.0 g, mp 222-223°C. A portion (5.0 g) was transmitted to WRAIR on January 15, 1997 (NJ24-87-2).

Anal.

| | | <u>C</u> | <u>H</u> | <u>N</u> |
|------------|--|----------|----------|----------|
| Calc'd for | $\text{C}_{19}\text{H}_{12}\text{Cl}_3\text{NO}$ | 60.59 | 3.21 | 3.72 |
| | Found | 60.35 | 3.29 | 3.67 |

Spectral Data

Infrared (KBr)

Major bands: 3430, 3060, 1690, 1610, 1590, 1555,
1475, 1450, 1430, 1395, 1370, 1300,
1270, 1230, 1205, 1190, 1130, 1100,
1070, 1050, 1015, 930 cm^{-1} .

Ultraviolet (CHCl_3)

λ_{max} 252 nm ($\log \epsilon$ 4.75); 293 nm (3.91).

Nuclear Magnetic Resonance (TFA-d)

δ 9.70 (s, 1, H-9); 8.45 (s, 1, H-8); 8.32 (m, 2,
H-5 and H-6); 7.53 (d, 1, C-3'), 7.37 (m, 1, C-5');
7.34 (d, 1, C-6'); 4.36 (m, 1, C-3); 3.97 (m, 2,
C-4); 3.39 (m, 2, C-2).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; visulization - UV.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----------------------|-----------------|----------------|
| Hexane-acetone (4:1) | 0.36 | Single spot |
| Hexane-EtOAc (2:1) | 0.74 | Single spot |

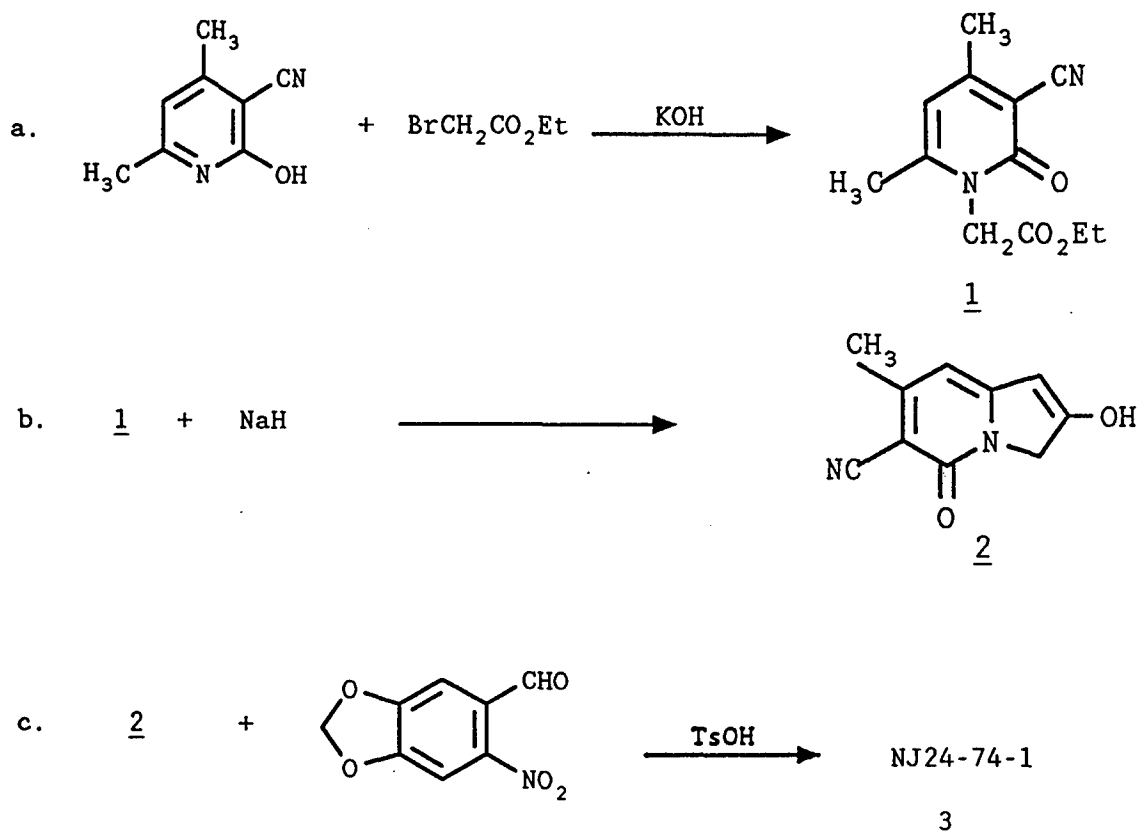
Source of Material

- | | | |
|----|--|----------------------------|
| 1. | 5-Chloro-2-nitrobenzaldehyde | Aldrich Chemical Co., Inc. |
| 2. | $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ | Sigma |
| 3. | CH_2Cl_2 | J.T. Baker Chemical Co. |
| 4. | Na_2SO_4 | J.T. Baker Chemical Co. |
| 5. | 5-(2,4-Dichlorophenyl)- cyclohexane-1,3-dione | Starks Associates, Inc. |
| 6. | Toluene | J.T. Baker Chemical Co. |
| 7. | <i>p</i> -Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 8. | Methanol | J.T. Baker Chemical Co. |
| 9. | Acetic acid | J.T. Baker Chemical Co. |

4. Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine (5)

The product(s) were prepared by the following sequence of reactions. The material was not fully characterized.

Reaction Sequence:



Experimental

Acetic acid, 2-[3-cyano-4,6-dimethyl-2-pyridone-1-yl]-ethyl ester (1)

To a solution of 3-cyano-4,6-dimethyl-2-hydroxypyridone (5.0 g, 3.4 mmol) and KOH (2.2 g of 85%, 3.3 mmol) in 2-methoxyethanol (50 mL) was added ethyl bromoacetate (6.0 g, 3.6 mmol), and the mixture was heated under reflux for 70 min then cooled. The solid was filtered off, washed with

2-methoxyethanol then discarded. To the filtrate was added conc. NH_4OH (0.8 mL) and H_2O (175 mL). The mixture was stored for 20 min then filtered. The collected solid (3.1 g, 39.2%) was suitable for further transformation. Additional product containing some starting material (2.2 g) was obtained from the filtrate.

Indolizine, 6-cyano-2,5-dihydroxy-7-methyl- (2)

Sodium hydride (0.52 g of 60%, 13 mmol) was added to THF (250 mL), and the suspension was cooled in an ice bath. To this was added 1 (3.0 g) and the mixture was stirred at RT, under an argon atmosphere, for 20 h then poured into H_2O (400 mL) and acidified with HCl. The solid that separated was collected on a filter, washed with H_2O (2 x 50 mL), then dried; yield 2.1 g (87.1%). The material was suitable for further transformation.

Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine (3)

To dry toluene (2 L) was added 2 (1.0 g, 5.3 mmol), 6-aminopiperonal (1.0 g, 6.1 mmol) and *p*-toluenesulfonic acid (200 mg, 1.1 mmol) and the mixture was heated under reflux overnight. The apparatus was equipped with a Dean Stark trap to collect any water formed during the reaction. The mixture was cooled and the solvent was removed in vacuo. The residue was purified by chromatography using CH_2Cl_2 -MeOH (3:1) as the eluent. Fractions containing product were combined then concentrated in vacuo. The residue was heated in 150 mL of 2-methoxyethanol, the insolubles were filtered off, and the filtrate concentrated in vacuo. The residue was triturated with Et_2O (50 mL) then dried; yield 0.9 g. A portion (200 mg) was transmitted to WRAIR on January 31, 1997 (Lot No. NJ24-74-1).

Spectral Data

Infrared (Nujol)

Major bands: 2900, 2840, 2200, 1640, 1590, 1520,
1490, 1460, 1370, 1350, 1270, 1240,
1025, 930, 860 cm^{-1} .

Nuclear Magnetic Resonance (TFA-d)

δ 11.50 (TFA); 9.29 (s, 1); 7.66 (s, 2); 7.48 (s, 1,
6.48 (s, 2); 5.96 (d, 1, $J=6.1$ Hz); 2.83 (s, 3).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

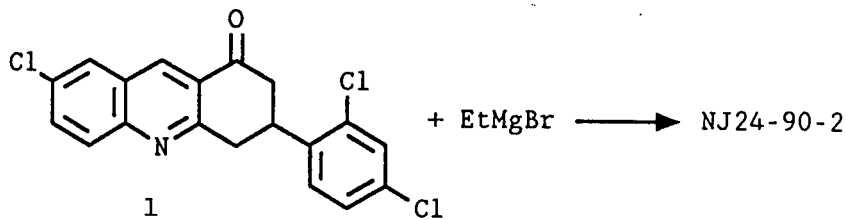
| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|---|-----------------|----------------|
| 1. | Hexane-acetone (6:4) | 0.20 | Homogeneous |
| 2. | CH_2Cl_2 - CH_3OH (9:1) | 0.59 | Homogeneous |

Source of Materials

| | | |
|----|--|----------------------------|
| 1. | 3-Cyano-4,6-dimethyl-2-hydroxypyridine | Aldrich Chemical Co., Inc. |
| 2. | KOH | Aldrich Chemical Co., Inc. |
| 3. | 2-Methoxyethanol | Aldrich Chemical Co., Inc. |
| 4. | Ethyl bromoacetate | Aldrich Chemical Co., Inc. |
| 5. | NaH | Aldrich Chemical Co., Inc. |
| 6. | Toluene | J.T. Baker Chemical Co. |
| 7. | <i>p</i> -Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 8. | MeOH | J.T. Baker Chemical Co. |

5. Product(s) obtained by reaction EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2H)-acridinone

Reaction Sequence:



Experimental

A 3M solution of EtMgBr in ether (0.5 mL, 1.5 mmol) diluted with THF (100 mL) was cooled and ketone 1 (please refer to p. 10, this report) was added. The mixture was allowed to warm to room temperature, refluxed for 2 h, then cooled. The mixture was quenched with H₂O (5 mL) then acidified with HCl. The solvent was removed in vacuo and the residue was dissolved in CH₂Cl₂ (100 mL). The solution was washed with H₂O (2 x 25 mL), dried, then concentrated to a solid (0.5 g). The material was chromatographed on a column of SiO₂ using hexane-EtOAc (2:1) as the eluent. Fractions 9-20 were combined then concentrated to give 300 mg of partially purified material. A portion of this material (150 mg) was applied to a preparative TLC plate and eluted with the same eluent. The band corresponding to the lower spot was collected then extracted with MeOH; yield 25 mg. A portion (15 mg) was transmitted to WRAIR on January 31, 1997 (Lot No. NJ24-90-2).

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

The spectrum appears to indicate the presence of many materials.

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|----------------------|-----------------|----------------|
| 1. | Hexane-EtOH (2:1) | 0.43 | single spot |
| 2. | Hexane-acetone (4:1) | 0.14 | single spot |

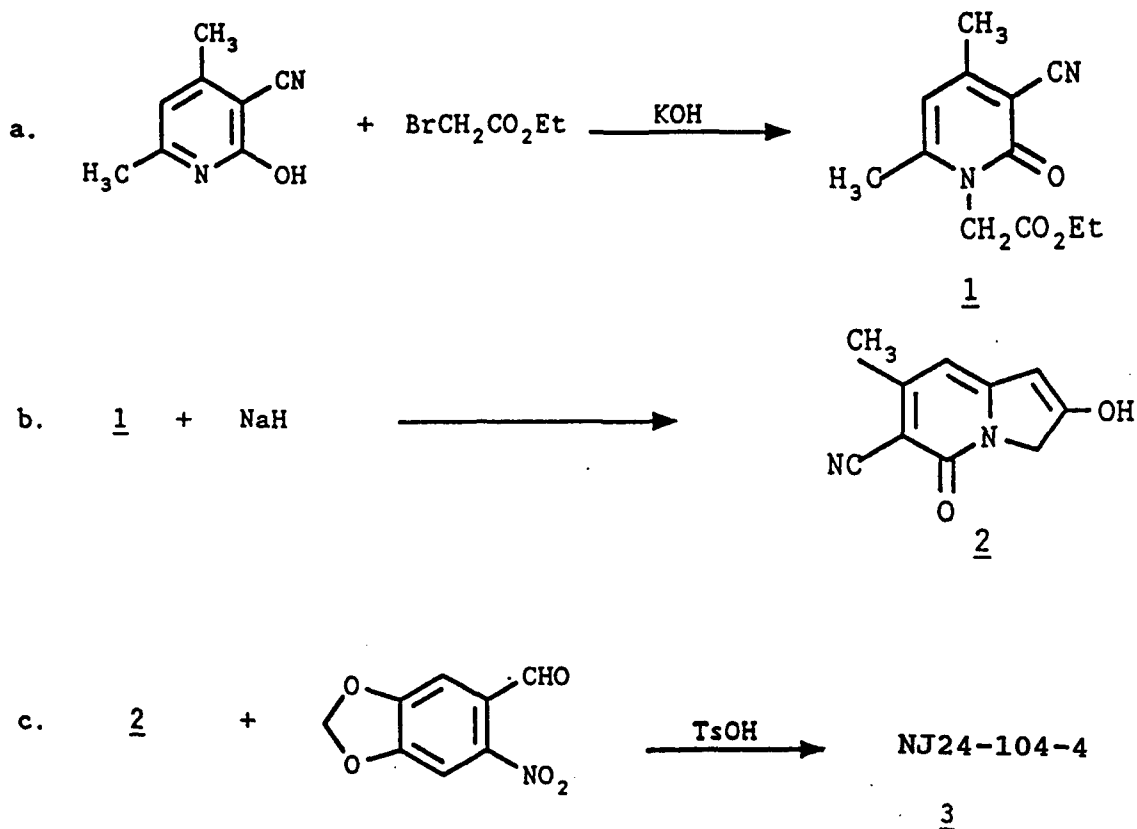
Source of Materials

| | | |
|----|---------------------------------|----------------------------|
| 1. | EtMgBr | Aldrich Chemical Co., Inc. |
| 2. | Ether | Fisher Scientific |
| 3. | THF | Aldrich Chemical Co., Inc. |
| 4. | HCl | J.T. Baker Chemical Co. |
| 5. | CH ₂ Cl ₂ | J.T. Baker Chemical Co. |
| 6. | SiO ₂ | EM Laboratories |
| 7. | Hexane | J.T. Baker Chemical Co. |
| 8. | EtOAc | J.T. Baker Chemical Co. |

6. Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine (3)

The product(s) were prepared by the following sequence of reactions. The material was not fully characterized.

Reaction Sequence:



Experimental

Acetic acid, 2-[3-cyano-4,6-dimethyl-2-pyridone-1-yl]-ethyl ester (1)

To a solution of 3-cyano-4,6-dimethyl-2-hydroxypyridine (5.0 g, 3.4 mmol) and KOH (2.2 g of 85%, 3.3 mmol) in 2-methoxyethanol (50 mL) was added ethyl bromoacetate (6.0 g, 3.6 mmol), and the mixture was heated under reflux for 70 min then cooled. The solid was filtered off, washed with

2-methoxyethanol then discarded. To the filtrate was added conc. NH_4OH (0.8 mL) and H_2O (175 mL). The mixture was stored for 20 min then filtered. The collected solid (3.1 g, 39.2%) was suitable for further transformation. Additional product containing some starting material (2.2 g) was obtained from the filtrate.

Indolizine, 6-cyano-2,5-dihydroxy-7-methyl- (2)

Sodium hydride (0.52 g of 60%, 13 mmol) was added to THF (250 mL), and the suspension was cooled in an ice bath. To this was added 1 (3.0 g) and the mixture was stirred at RT, under an argon atmosphere, for 20 h then poured into H_2O (400 mL) and acidified with HCl. The solid that separated was collected on a filter, washed with H_2O (2 x 50 mL), then dried; yield 2.1 g (87.1%). The material was suitable for further transformation.

Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine (3)

To dry toluene (1 L) was added 2 (1.8 g, 9.56 mmol), 6-aminopiperonal (1.8 g, 10.90 mmol), *p*-toluenesulfonic acid (100 mg, 0.52 mmol) and glacial acetic acid (3.6 g, 59.9 mmol). The reaction mixture was heated under reflux for 4 h. The apparatus was equipped with a Dean Stark trap to collect any water formed during the reaction. The mixture was cooled and the solvent was removed in vacuo. The residue was washed with CH_2Cl_2 (100 mL), hot 2-methoxyethanol (200 mL) and then boiling acetic acid (200 mL). The insolubles were dried in vacuo at 90°C to obtain 2.8 g of 3 in 92.4% yield. A portion (100 mg) was transmitted to WRAIR on April 15, 1997 (Lot No. NJ24-104-4).

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> |
|---------------------------------|----------|----------|----------|
| Calc'd for $C_{18}H_{11}N_3O_3$ | 68.14 | 3.50 | 13.24 |
| Found | 67.76 | 3.76 | 13.15 |

Spectral Data

Infrared (Nujol)

Major bands: 2900, 2840, 2200, 1640, 1590, 1520,
1490, 1460, 1370, 1350, 1270, 1240,
1025, 930, 860 cm^{-1} .

Nuclear Magnetic Resonance (TFA-d)

δ 11.50 (TFA); 9.21 (s, 1); 7.57 (d, 2); 7.40 (s, 1,
6.40 (s, 2); 5.08 (d, 1, J=6.1 Hz); 2.74 (s, 3).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|-----------------------------|-----------------|----------------|
| 1. | Hexane-acetone (6:4) | 0.20 | Homogeneous |
| 2. | CH_2Cl_2 - CH_3OH (9:1) | 0.59 | Homogeneous |

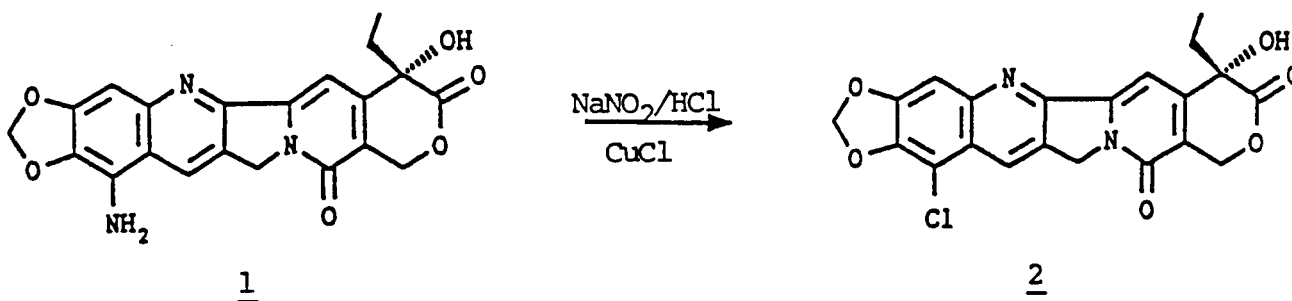
Source of Materials

- | | | |
|----|--|----------------------------|
| 1. | 3-Cyano-4,6-dimethyl-2-hydroxypyridine | Aldrich Chemical Co., Inc. |
| 2. | KOH | Aldrich Chemical Co., Inc. |
| 3. | 2-Methoxyethanol | Aldrich Chemical Co., Inc. |
| 4. | Ethyl bromoacetate | Aldrich Chemical Co., Inc. |
| 5. | NaH | Aldrich Chemical Co., Inc. |
| 6. | Toluene | J.T. Baker Chemical Co. |
| 7. | <i>p</i> -Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 8. | MeOH | J.T. Baker Chemical Co. |

7. (S)-15-Chloro-7-ethyl-7-hydroxy-10H-1,3-dioxolo[4,5-g]-pyrano[3',4',:6,7]indolizino[1,2-b]quinoline-8,11-(7H,13H)-dione
Syn.: 9-Chloro-10,11-(methylenedioxy)-20(S)-camptothecin (2)

The target compound was prepared by the following reaction.

Reaction Sequence:



Experimental⁵

9-Amino-10,11-(methylenedioxy)-20(S)-camptothecin (1)

Please refer to Starks Associates, Inc., Annual Report dated January 1996, Contract DAMD17-93-C-3003, p. 92.

9-Chloro-10,11-(methylenedioxy)-20(S)-camptothecin (2)

9-Amino-10,11-(methylenedioxy)-20(S)-camptothecin (1) (5.2 g, 12.76 mmol) was added to stirred conc. HCl (275 mL) at -10°C. To the resulting yellow solution was added NaNO₂ (1.24 g, 17.98 mmol, in 20 mL H₂O) over 2 min. The reaction was stirred at this temperature for 5 min. then CuCl (2.5 g, 25.25 mmol) was added in small portions over 3 min. During

this time foaming occurred and the reaction changed to a dark brown color. The cooling bath was removed, and after 1 h the reaction was poured into 1.5 kg of ice. The precipitate was collected and dried in vacuo at 90°C. The filtrate was extracted with CH₂Cl₂ (6 x 500 mL). The organic phase was washed with H₂O (2 x 200 mL), dried over Na₂SO₄ and concentrated in vacuo. The solid obtained was combined with the previously precipitated material to obtain 5.3 g of very crude product. This was chromatographed on a 2 kg silica gel column using CH₂Cl₂/MeOH (95:5) as the eluent to obtain 3.7 g of material with less impurity. Additional material (2.4 g) of similar purity was obtained in a similar fashion. These two lots were combined and washed with boiling methanol (1 L). The solid collected (4.8 g), was extracted with boiling CHCl₃ in a Soxhlet extraction apparatus. Removal of CHCl₃ in vacuo produced 4.0 g of 2; mp >300°C. A portion (3.9 g) was transmitted to WRAIR on June 6, 1997 (Lot No. NJ24-108-1).

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> | <u>Cl</u> |
|--|----------------|--------------|--------------|-----------|
| Calc'd for C ₂₁ H ₁₅ ClN ₂ O ₆ • 0.75 HCl | 55.53 | 3.49 | 6.17 | 13.66 |
| Found | 55.31 55.28 | 3.54 3.61 | 6.01 5.98 | 13.43 |

Spectral Data

Infrared (KBr)

Major bands: 3400, 2950, 2900, 1740, 1655, 1610, 1585, 1490, 1445, 1380, 1240, 1150, 1110, 1040, 940 cm⁻¹.

Ultraviolet (DMSO)

λ_{max} 260 nm (sh, log ε 4.37); 305 nm (3.93)
374 nm (4.41); 393 nm (4.48).

Nuclear Magnetic Resonance (DMSO-d₆)

δ 8.76 (s, 1, H-7); 7.60 (s, 1, H-12); 7.32 (s, 1, H-14); 6.52 (s, 1, OH); 6.45 (s, 2, OCH₂O); 5.47 (s, 2, H-17); 5.32 (s, 2, H-5); 1.91 (m, 2, H-19); 0.93 (t, 3, H-18).

Optical Rotation

$[\alpha]_D^{20} = -27.18^\circ\text{C}$ (C 0.08, DMSO)

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|---|-----------------|----------------|
| 1. CH ₂ Cl ₂ -acetone-CH ₃ OH (75:20:5) | 0.73 | Streaks |

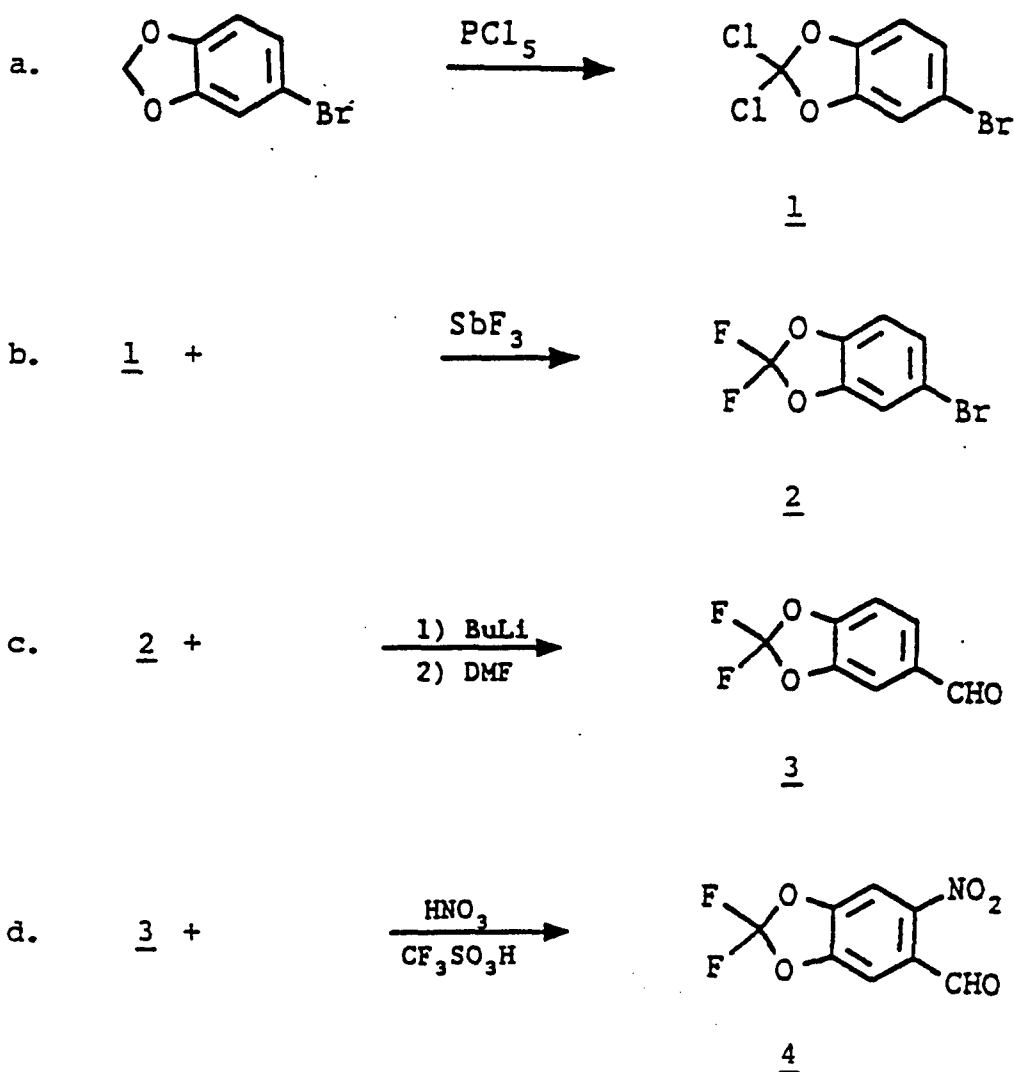
Source of Materials

| | | |
|----|---|----------------------------|
| 1. | 9-Amino-10,11-(methylenedioxy)- 20(S)-camphothecin | Starks Associates, Inc. |
| 2. | HCl | J.T. Baker Chemical Co. |
| 3. | Sodium nitrite | Aldrich Chemical Co., Inc. |
| 4. | CuCl | Aldrich Chemical Co., Inc. |
| 5. | CH ₂ Cl ₂ | J.T. Baker Chemical Co. |
| 6. | Silica gel | EM Laboratory |
| 7. | MeOH | J.T. Baker Chemical Co. |
| 8. | CHCl ₃ | Aldrich Chemical Co., Inc. |

8. (S)-7-Ethyl-7-hydroxy-10H-2,2-difluoro-1,3-dioxolo-[4,5-g]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-8,11(7H,13H)-dione
Syn:10,11-(Difluoromethylenedioxy)-20(S)-camptothecin (7)

The target compound (7) was prepared by the following sequence of reactions.

Reaction Sequence:



7

Experimental

3,4-(Dichloromethylenedioxy)bromobenzene (1)³

A stirred mixture of phosphorus pentachloride (80 g, 0.38 mol) and 3,4-(methylenedioxy)bromobenzene (22.4 g, 0.11 mol) was heated at 80-85°C for 3.5 h. Distillation gave 26 g (87.5%) of 1, bp 116-118°/4.5 mm; literature³ bp 107-109°/4 mm. Additional product (61.7 g) was obtained from a scouting run and a larger run. The material was suitable for further transformation.

Anal.

| | | | Total halogen calculated as | | |
|------------|--|----------|--------------------------------|-----------|-----------|
| | | <u>C</u> | <u>H</u> | <u>Br</u> | <u>Cl</u> |
| Calc'd for | C ₇ H ₃ Cl ₂ BrO ₂ | 31.23 | 1.12 | 88.81 | 39.40 |
| | Found | 31.08 | 1.17 | 88.76 | 39.38 |

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.21 (m, 2, H's at C-2 & C-5); 6.93
(d, 1, J=9.7 Hz, H at C-6).

3,4-(Difluoromethylenedioxy)bromobenzene (2)³

Compound 1 (26.0 g, 96.3 mmol) was heated with SbF₃ (26.0 g, 145.5 mmol) at 20 mm to give 20.8 g of crude product. The material was purified by passing the crude material through a pad of silica gel (100 g) using ether as the eluent; yield 19.3 g (84.6%). Additional product (52.65 g) was obtained from a scouting run and a larger run. The product was suitable for further transformation.

Anal.

| | <u>C</u> | <u>H</u> |
|--|--------------|------------|
| Calc'd for C ₇ H ₃ BrF ₂ O ₂ | 35.47 | 1.28 |
| Found | 36.52, 36.53 | 1.64, 1.67 |

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.20 (m, 2, H's at C-2 & C-5); 6.92 (d, 1, J=8.7 Hz, H at C-6).

3,4-(Difluoromethylenedioxy)benzaldehyde (3)³

To a stirred, cooled (-40°C) solution of 2 (50 g, 0.21 mol) in Et₂O (200 mL) was added butyllithium (145 mL of 1.6 M solution in hexane, 0.232 mol), dropwise. After the addition the mixture was stirred at -40°C for 1 h then DMF (48 mL, 0.61 mol) was added, dropwise. The mixture was stirred for 2.5 h at ambient temperature, cooled, and saturated ammonium chloride solution (100 mL) was added and the mixture was stirred for 10 min then extracted with ether (2 x 200 mL). The extracts were combined, washed with brine (2 x 50 mL), dried (MgSO₃), then concentrated in vacuo to an oil. The oil was distilled to yield 23 g (59%) of 3, bp 117-120°/25 mm; literature³ bp 103-105/20 mm. Additional product (7.1 g) was obtained from a scouting run. The material was suitable for further transformation.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 9.93 (s, 1, CHO); 7.61 (dd, 1, J=8.1, 1.8 Hz);
H at C-6); 7.61 (d, 1, J=2.0 Hz, H at C-2); 7.24
(d, 1, J=8.1 Hz, H at C-5).

3,4-(Difluoromethylenedioxy)-6-nitrobenzaldehyde (4)

To a solution of trifluoromethanesulfonic acid (8 mL) in anhydrous CH₂Cl₂ (100 mL) was added 90% nitric acid (2.5 mL) (this nitration methodology⁶ has been used for other compounds). A white solid separated from the solution. The suspension was cooled to -60°C and 3,4-(difluoromethylene-dioxy)benzaldehyde (1.7 g, 9.1 mmol) was added, dropwise. The mixture was stirred at -60°C for 2 h, at 0°C for 1.5 h, then

stored in a refrigerator, overnight. The cool mixture was poured over ice (50 g) and extracted with CH_2Cl_2 (2 x 200 mL). The extracts were washed with water (2 x 200 ml), dried (MgSO_4), then concentrated in vacuo to an oil. The oil was purified by chromatography on a column of silica gel (100 g) using hexane- CH_2Cl_2 -EtOAc (10:1:1) as the eluent. Fractions containing pure product were combined and concentrated to give 1.52 g (72.5%) of 4, after crystallization from hexane/ether; mp 35-36°C. Additional product (20 g) was obtained from two larger runs. The material was suitable for further transformation.

Anal.

| | | <u>C</u> | <u>H</u> | <u>N</u> |
|------------|---|----------|----------|----------|
| Calc'd for | $\text{C}_8\text{H}_3\text{F}_2\text{NO}_5$ | 41.57 | 1.30 | 6.06 |
| | Found | 41.46 | 1.38 | 6.04 |

Spectral Data

FT-Infrared (KBr)

ν 3126.6, 3073.6, 2920.4, 1701.8, 1621.9, 1536.5, 1489.0, 1424.5, 1343.4, 1273.2, 1252.5, 1216.1, 1178.5, 1129.2, 1068.6, 892.8, 856.1, 829.2, 802.2, 752.9, 710.0, 669.0 603.3, 450.6 cm^{-1} .

Nuclear Magnetic Resonance ($\text{DMSO}-d_6$)

δ 10.13 (s, 1, CHO); 8.37 (s, 1, H-5); 7.91 (s, 1, H-2).

6-Amino-3,4-(difluoromethylenedioxy)benzaldehyde (5)

To a solution of ferrous sulfate (8.0 g, 28.8 mmol) in boiling H₂O (40 mL) was added, dropwise, a solution of 4 (0.7 g, 3 mmol) in EtOAc (10 ml) then ammonium hydroxide (10 mL), in small portions until the solution remained alkaline. The mixture was refluxed for 5 min, cooled, diluted with EtOAc (50 ml), then filtered. The aqueous portion was washed with brine (2 x 10 ml), dried (MgSO₄) then concentrated to an oil. The oil was purified by chromatography on silica gel using hexane-EtOAc-CH₂Cl₂ (10:1:1) as the eluent to yield 200 mg (33.2%) of 5, mp 80-81°C⁴. Additional product (4.76 g), was obtained from larger runs. The material was suitable for further transformation.

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> |
|---|----------|----------|----------|
| Calc'd for C ₈ H ₅ F ₂ NO ₃ | 47.77 | 2.51 | 6.96 |
| Found | 47.79 | 2.51 | 6.98 |

Spectral Data

Nuclear Magnetic Resonance (DMSO-d₆)

δ 9.74 (s, 1, CHO); 7.60 (s, 1, H-2); 7.53 (s, 2H, NH₂); 6.76 (s, 1, H-5).

(S)-7-Ethyl-7-Hydroxy-10H-2,2-difluoro-1,3-dioxolo[4,5-g]-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-8,11(7H,13H)-dione (7)

A stirred mixture of 5 (200 mg, 1 mmol), (S)-4-ethyl-7,8-dihydro-4-hydroxy-1H-pyrano[3,4-f]indolizine-3,6,10-(4H)-trione (265 mg, 1 mmol), toluene (1.5 mL) and glacial acetic

acid (0.5 mL) was heated until solution was obtained (~60°C). *p*-Toluenesulfonic acid (50 mg) was added, and the mixture was heated under reflux for 17 h, cooled, concentrated to a smaller volume then diluted with ethanol. The precipitate was collected and recrystallized (EtOH); yield 120 mg, mp 269-270°C. Additional product (40 mg) was obtained from mother liquor by chromatography on silica gel using EtOAc-hexanes (10:1) as the eluent; total yield 160 mg (37.4%). Additional product (885 mg) was obtained from three additional runs. The two lots were combined then recrystallized from EtOH and EtOAc, yield 860 mg; mp 270-271°C⁴. A portion (700 mg) was transmitted to WRAIR on June 25, 1997 (Lot No. NJ29-14-2).

Anal.

| | | <u>C</u> | <u>H</u> | <u>N</u> |
|------------|--|----------|----------|----------|
| Calc'd for | C ₂₁ H ₁₄ F ₂ N ₂ O ₆ | 58.88 | 3.29 | 6.54 |
| | Found | 58.39 | 3.42 | 6.43 |

Spectral Data

FT-Infrared (KBr)

ν 3420.2 (OH), 3097.2, 3067.0, 2976.0, 2940.0, 2882.5, 1749.0 (C=O), 1660.8, 1618.0, 1600.6, 1558.8, 1506.3, 1464.6, 1386.8, 1239.9, 1156.1, 1108.8, 1035.4, 1002.0, 927.6, 912.4, 859.3, 807.8, 708.8, 667.3, 583.4, 483.8 cm⁻¹.

Ultraviolet (DMSO)

λ_{max} 292 nm (log ε 3.44); 326 nm (sh, 3.17); 369 nm (3.83); 385 nm (3.78).

Mass Spectrum (Acq Method)

m/z 428 (M, abund 100), 429 (M+1, abund 38).

Nuclear Magnetic Resonance (DMSO-d₆)

δ 8.67 (s, 1H, H-7); 8.11 (s, 1H, H-12); 8.09 (s, 1H, H-9); 7.29 (s, 1H, H-14); 6.51 (s, 1H, OH); 5.40 (s, 2H, H-17); 5.25 (s, 2H, H-5); 1.83 (m, 2H, CH₂-CH₃); 0.85 (t, 3H, J₁=7.2 Hz, J₂=7.4 Hz, CH₂-CH₃).
D₂O exchanges proton at 6.51 ppm.

Optical Rotation

$[\alpha]_D^{20} = -17.5$ [C 0.104, DMSO].

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|--|-----------------|----------------|
| 1. | EtOAc-hexane (10:1) | 0.43 | Homogeneous |
| 2. | EtOAc-CH ₂ Cl ₂ -hexane (1:1:1) | 0.67 | Homogeneous |
| 3. | EtOAc | 0.32 | Homogeneous |

Source of Materials

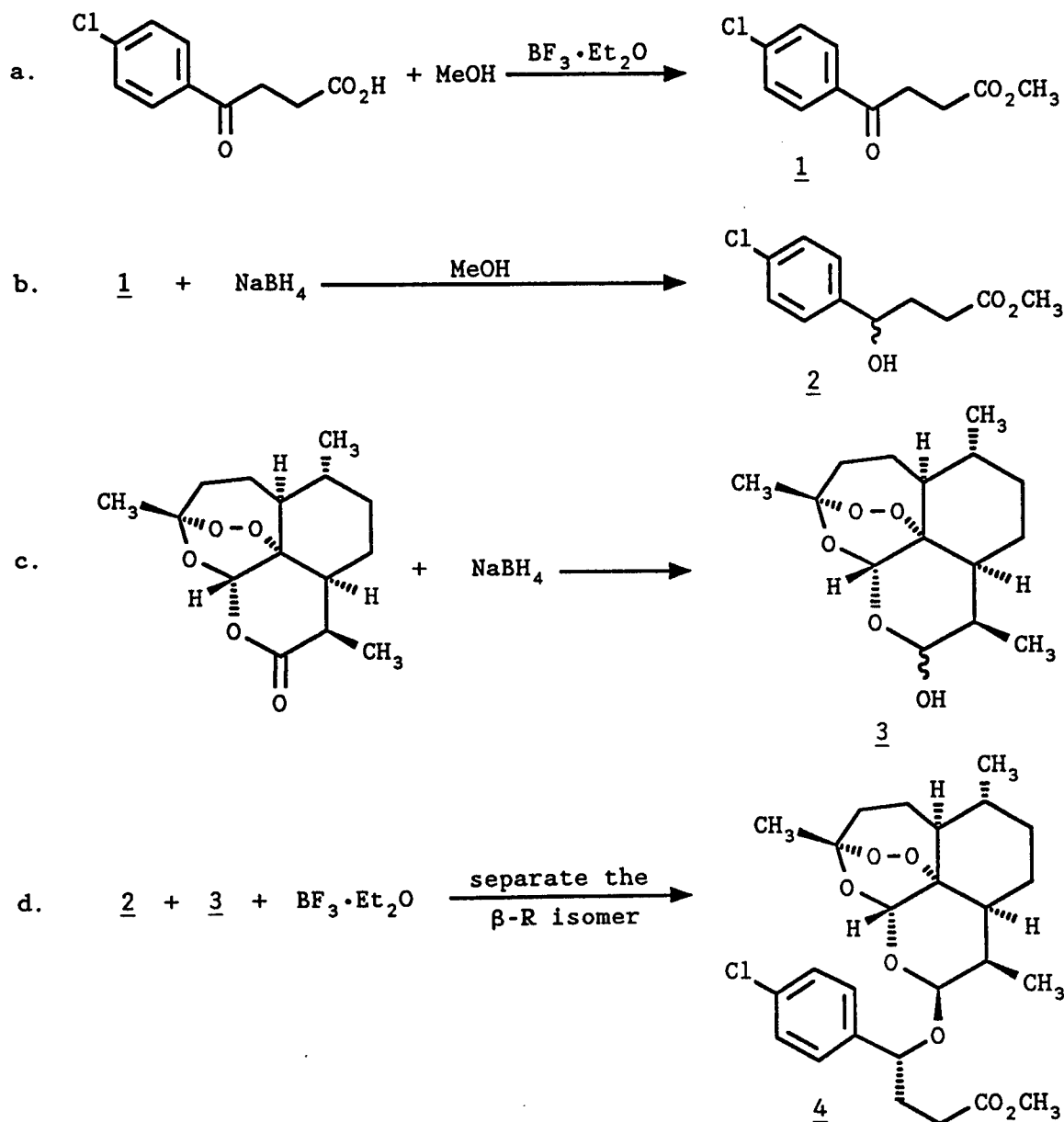
| | | |
|----|---|----------------------------|
| 1. | Phosphorus pentachloride | Aldrich Chemical Co., Inc. |
| 2. | 3,4-(Methylenedioxy)bromobenzene | Aldrich Chemical Co., Inc. |
| 3. | 3,4-(Dichloromethylenedioxy)-bromobenzene | Starks Associates, Inc. |
| 4. | Antimony trifluoride | Aldrich Chemical Co., Inc. |
| 5. | Silica gel | EM Laboratories |
| 6. | 3,4-(Difluoromethylenedioxy)-bromobenzene | Starks Associates, Inc. |
| 7. | Ether | Fisher Scientific |
| 8. | Butyllithium | Aldrich Chemical Co., Inc. |
| 9. | DMF | Aldrich Chemical Co., Inc. |

| | | |
|-----|---|----------------------------|
| 10. | NH_4Cl | Aldrich Chemical Co., Inc. |
| 11. | Trifluoromethanesulfonic acid | Aldrich Chemical Co., Inc. |
| 12. | CH_2Cl_2 | J.T. Baker Chemical Co. |
| 13. | 3,4-(Difluoromethylenedioxy)-benzaldehyde | Starks Associates, Inc. |
| 14. | Nitric acid | J.T. Baker Chemical Co. |
| 15. | Hexane | J.T. Baker Chemical Co. |
| 16. | EtOAc | J.T. Baker Chemical Co. |
| 17. | Ferrous sulfate $\cdot 7\text{H}_2\text{O}$ | J.T. Baker Chemical Co. |
| 18. | 3,4-(Difluoromethylenedioxy)-6-nitrobenzaldehyde | Starks Associates, Inc. |
| 19. | Ammonium hydroxide | J.T. Baker Chemical Co. |
| 20. | 6-Amino-3,4-(difluoromethylene-dioxy)benzaldehyde | Starks Associates, Inc. |
| 21. | (S)-4-Ethyl-7,8-dihydro-4-hydroxy-1H-pyrano[3,4-f]-indolizine-3,6,10(4H)-trione | WRAIR (BN78921) |
| 22. | Toluene | J.T. Baker Chemical Co. |
| 23. | Acetic acid | J.T. Baker Chemical Co. |
| 24. | p-Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 24. | Ethanol | U.S. Industrial |

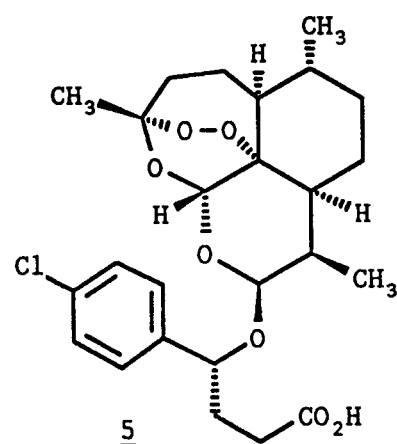
9. Butyric acid, 4-(4-chlorophenyl)-4(R)-[10(β)-dihydro-artemisininoxy]- (5)

The target compound 5 was prepared by the following sequence of reactions.

Reaction Sequence:



e. 4 + KOH



Experimental

Propionic acid, 3-(4-chlorobenzoyl)- methyl ester (1)

To a solution of 3-(4-chlorobenzoyl)propionic acid (50.0 g, 0.235 mol) in CH_3OH (500 mL) was added, dropwise, boron trifluoride diethyl etherate (26.8 g, 30 mL, 0.188 mol), and the mixture was refluxed for 24 h. TLC (ethyl acetate-hexane (1:4 v/v)) indicated a complete reaction. Approximately half of the solvent was removed under reduced pressure, and the residue was poured into 1500 mL of water. The solid that formed was collected on a filter, washed with water then dissolved in ethyl acetate. The EtOAc solution was washed with sodium bicarbonate, dried (Na_2SO_4) then concentrated in vacuo to an oil which crystallized on standing, yield 52.5 g (98.5%). Additional product was obtained from other reactions for a total of 136.1 g (98.2% overall). The material was suitable for further transformation.

Spectral Data

Nuclear Magnetic Resonance (CDCl_3)

δ 7.92 (d, 2, aromatic H's at 2 & 6); 7.44 (d, 2, aromatic H's at 3 & 5); 3.71 (s, 3, OCH_3); 3.28 and 2.77 (t, 2, $J = 6.6$ Hz, CH_2CH_2).

Butyric acid, 4-(4-chlorophenyl)-4-hydroxy-
methyl ester (2)

To a cooled (0-5°C) solution of 1 (52.0 g, 0.229 mol) in anhydrous methanol (540 mL) was added sodium borohydride (9 g, 0.24 mol) in small portions, over 50 min. Progress of the reaction was monitored by silica gel TLC (EtOAc:hexanes 1:2 (v/v)) until the reaction was complete (~1.5 h). The reaction was quenched by addition of 30% AcOH in CH₃OH until pH 6. The solution was evaporated to dryness, and the oil was dissolved in EtOAc (500 mL). The solution was washed with aq. sodium bicarbonate (2 x 150 mL), H₂O (2 x 100 mL), dried (Na₂SO₄), then evaporated to dryness. The crude product was purified by column chromatography on silica gel (1 kg) using EtOAc:hexane (1:2 v/v) as the eluent. Fractions containing product were combined then concentrated in vacuo to give 42.0 g (79.8%) of 2 as an oil. Additional product was obtained from other reactions for a total of 86.6 g (77.7% overall). The material was suitable for further transformation.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.34-7.25 (m, 4, aromatic H's); 4.69
(t of d, 1, J= 3.7 Hz, 5.9 Hz, -CH); 3.63
(s, 3, OCH₃); 2.39 (t, 2, J= 6.7 Hz, COCH₂);
2.00 (d of t, J= 4.0 Hz, 6.9 Hz, CHCH₂).

Dihydroartemisinin (3)

To a cooled (0°C), stirred, solution of artemisinin (37.0 g, 0.131 mol) in CH₃OH (1.5 L) was added solid NaBH₄ (37.0 g, 1.96 mol) in portions. The mixture was kept at 0-5°C for 3 h then quenched by dropwise addition of AcOH (63 mL) in CH₃OH (310 mL). The mixture was stirred for 15 min. then concentrated in vacuo (bath temperature <20°C) to a residue. The residue was extracted with CHCl₃ (2 x 500 mL). The extracts were combined, washed with a saturated sodium bicarbonate solution (2 x 250 mL), brine (2 x 250 mL), dried (MgSO₄) then concentrated in vacuo (<20°C) to give 36.8 g (98.7%) of 3. Additional product was obtained from an identical reaction for a total of 71.5 g of 3 (96.0% overall).

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 5.49 (s, 1, H at C₁₂ for β isomer); 5.34 (s 1, H at C₁₂ for α-isomer); 5.20 (d, 1, H at C₁₀ for β isomer) 4.45 (m, 1, H at C₁₀ for α-isomer).

Butyric acid, 4-(4-chlorophenyl)-4(R)-[10(β)-dihydro-artemisininoxy]- methyl ester (4)

To a suspension of 2 (42.0 g, 0.184 mol) and 3 (21.0 g, 0.074 mol) in ether (1 L) was added 10% boron trifluoride diethyl etherate (110 mL) (prepared by dilution of 11 mL of boron trifluoride diethyl etherate with 100 mL of ether), and the mixture was stored at RT overnight. The reaction was terminated by addition of a saturated sodium bicarbonate solution (500 mL). The organic layer was separated, washed

twice with H₂O, dried (Na₂SO₄), then evaporated to dryness. The gummy residue was purified by silica gel column chromatography using EtOAc:hexanes (1:4 v/v) as the eluent to give 17.0 g (46.4%) of 4*R*(β)diastereomer and 8.0 g (21.9%) of 4*S*(β)-isomer. Additional product was obtained from a scouting run and another reaction for a total of 38.1 g (50.5% overall) of 4. The material was suitable for further transformation.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.30-7.22 (m, 4H, aromatic H's); 5.47 (s 1, H at C₁₂); 4.82 (t, 1, *J*= 7.0 Hz, CHO); 4.59 (d, 1, *J*= 3.5 Hz, H at C₁₀); 3.65 (s, 3, OCH₃).

Butyric acid, 4-(4-chlorophenyl)-4(*R*)-[10(β)-dihydro-artemisininoxy]- (5)

A solution of 4 (18.0 g, 36.4 mmol) in 2.5 % KOH in methanol (540 mL) was stirred at RT for 2.5 days. The solution was diluted with H₂O (450 mL) and the methanol was removed in vacuo. The aqueous solution was basified to pH 9 with 5% sodium carbonate then twice extracted with ether. The ether extracts were discarded. The aqueous portion was acidified with diluted HCl then extracted with ether (3 x 700 mL). The organic portions were combined dried (Na₂SO₄) then concentrated in vacuo to give 5 (17.0 g, 97.1% yield). Additional product was obtained from another reaction for a total of 32 g of crude 5 (86.7% overall). Analytically pure product (25.2 g, 78.8% recovery) was obtained by recrystallizing the solid from hexanes/EtOAc; mp. 150-151°C;

literature⁷ mp 147°C. A portion (25.2 g) was transmitted to WRAIR on August 20, 1997 (Lot No. NJ29-36-2).

Anal.

| | <u>C</u> | <u>H</u> | <u>Cl</u> |
|--------------------------------|--------------|------------|-----------|
| Calc'd for $C_{25}H_{33}ClO_3$ | 62.42 | 6.91 | 7.37 |
| Found | 62.68, 62.61 | 6.93, 6.98 | 7.71 |

Spectral Data

FT-Infrared (KBr)

ν 2953.8, 2929.5, 2871.0, 2691.7, 2570.2, 1705.0, 1488.6, 1454.3, 1437.9, 1408.3, 1379.3, 1281.6, 1251.6, 1227.1, 1192.6, 1137.9, 1098.0, 1061.7, 1025.9, 990.2, 960.9, 940.5, 907.4, 876.2, 825.8, 679.6, 559.5, 540.1, 530.0, 483.9 cm^{-1} .

Ultraviolet (EtOH)

λ_{max} 260 nm (log ϵ 2.33); 266 nm (2.39);
270 nm (sh, 2.27).

Nuclear Magnetic Resonance (CDCl₃)

δ 7.30-7.23 (m, 4H, aromatic H's); 5.47 (s 1, H at C₁₂); 4.86 (t, 1, J= 6.5 Hz); 4.60 (d, 1, J= 3.4 Hz, H at C₁₀); 3.65 (s, 3, OCH₃).

Optical Rotation

$[\alpha]_D^{20} = +182.5^\circ$ (C= 1.006, CH₂Cl₂).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|--------------------|-----------------|----------------|
| 1. | EtOAc-hexane (1:2) | 0.25 | Homogeneous |
| 2. | EtOAc | 0.85 | Homogeneous |
| 3. | EtOAc-hexane (1:1) | 0.62 | Homogeneous |

Source of Materials

| | | |
|-----|---|----------------------------|
| 1. | Artemisinin | WRAIR |
| 2. | 3-(4-Chlorobenzoyl)- propionic acid | Aldrich Chemical Co., Inc. |
| 3. | NaBH ₄ | Aldrich Chemical Co., Inc. |
| 4. | BF ₃ · Et ₂ O | Aldrich Chemical Co., Inc. |
| 5. | CH ₃ OH | J.T. Baker Chemical Co. |
| 6. | Et ₂ O | Fisher Scientific |
| 7. | EtOAc | J.T. Baker Chemical Co. |
| 8. | Sodium bicarbonate | Aldrich Chemical Co., Inc. |
| 9. | Methyl 3-(4-chlorobenzoyl)- propionate | Starks Associates, Inc. |
| 10. | Methyl 4-(4-chlorophenyl)- 4-hydroxybutyrate | Starks Associates, Inc. |
| 11. | Silica gel | EM Laboratories |
| 12. | AcOH | J.T. Baker Chemical Co. |
| 13. | CHCl ₃ | Aldrich Chemical Co., Inc. |
| 14. | Dihydroartemisinin | Starks Associates, Inc. |
| 15. | Methyl 4-(4-chlorophenyl)- 4(R)-[10(β)-dihydroarte- misininooxybutyrate | Starks Associates, Inc. |

- | | | |
|-----|------------------|----------------------------|
| 16. | KOH | Aldrich Chemical Co., Inc. |
| 17. | Sodium carbonate | Aldrich Chemical Co., Inc. |
| 18. | HCl | J.T. Baker Chemical Co. |

10. Artemisinin

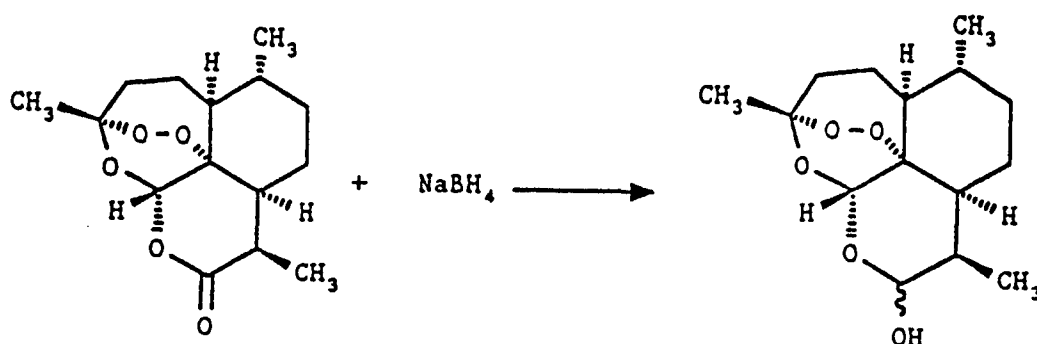
A portion of artemisinin (10 g), which we have originally received from the Institute of Chemistry, Hanoi was transmitted to WRAIR on September 18, 1997 (Lot No. NJ26-48-1).

Nuclear Magnetic Resonance (CDCl₃)

δ 5.92 (s, 1, OCHO); 3.10 (q, 1, CH₃CHCO);

11. Dihydroartemisinin (1)

Reaction Sequence



Experimental

To a cooled (0°C), stirred solution of artemisinin (75.0 g, 0.266 mol) in methanol (3.0 L) was added in small portions, sodium borohydride (75.0 g, 3.97 mol) during 1 h, keeping the reaction temperature at $0-5^\circ\text{C}$. The solution was stirred under argon at $0-5^\circ\text{C}$ until all starting material had reacted (~ 4 h), then neutralized with acetic acid (125 mL) in methanol (750 mL), the mixture was concentrated in vacuo to a solid (bath temperature $<20^\circ\text{C}$) and the solid was extracted with ethyl acetate (4×2.5 L). The extracts were combined and concentrated in vacuo to a solid. The material was triturated with hexane (940 mL); yield 63.6 g (84.1%) (75.0 g, 3.97 mol), m.p. $135-138^\circ\text{C}$, literature^{8a,8b} m.p. $153-154^\circ\text{C}$ (d). The product (10.0 g) was transmitted to WRAIR on November 10, 1997 (Lot No. NJ26-61-4).

Spectral Data:

Infrared (Nujol)

Major bands: 3375, 2900, 2830, 1710, 1565, 1450, 1375, 1302, 1275, 1222, 1200, 1185, 1170, 1155, 1125, 1085, 1055, 1028, 980, 928, 890, 870, 835, 815 cm^{-1} .

Ultraviolet (Ethanol)

No absorption at 5.3×10^{-2} g/L.

Nuclear Magnetic Resonance (CDCl₃)

δ 5.59 (s, 1, H at C₁₂ for β -isomer); 5.37 (s, 1, H at C₁₂ for α -isomer); 5.28 (d, 1, H at C₁₀ for β -isomer) 4.74 (d, 1, H at C₁₀ for α -isomer).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

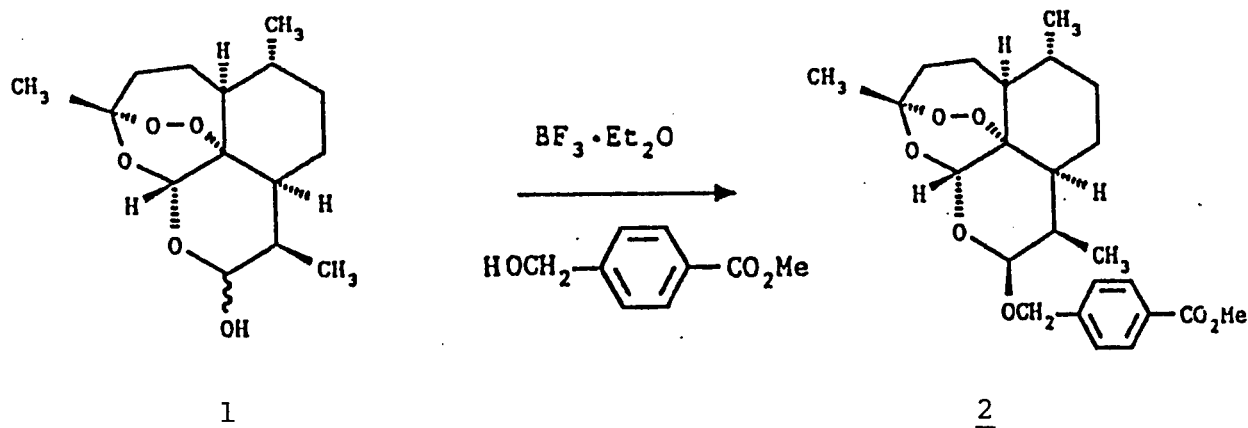
| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comments</u> |
|----|--------------------|-----------------|-------------------------|
| 1. | EtOAc-hexane (2:1) | 0.51 | baseline trace impurity |
| 2. | Hexane-EtOAc (2:1) | 0.14 | baseline trace impurity |

Source of Materials

| | | |
|----|-------------------|----------------------------|
| 1. | Artemisinin | WRAIR |
| 2. | Methanol | J.T. Baker Chemical Co. |
| 3. | NaBH ₄ | Aldrich Chemical Co., Inc. |
| 4. | Acetic acid | J.T. Baker Chemical Co. |
| 5. | Ethyl acetate | J.T. Baker Chemical Co. |
| 6. | Hexane | J.T. Baker Chemical Co. |

12. Artelinic acid, methyl ester (2)

Reaction Sequence



Experimental

Dihydroartemisinin (1)

Please refer to the preceding synthesis, this report.

Methyl 4-(10'-dihydroartemisininoxymethyl)benzoate (2)

To a stirred suspension of 1 (53.6 g, 0.188 mol) in ether (6.1 L), was added methyl 4-hydroxymethylbenzoate (107.5 g, 0.647 mol) followed by $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (27.0 mL, 0.219 mol). The resulting solution was stirred at room temperature for 24 h, washed with 5% NaHCO_3 (2 x 600 mL), H_2O (2 x 600 mL) dried with Na_2SO_4 , then concentrated in vacuo to an oil. This was chromatographed on a column of silica gel (2 kg) using hexane-ethyl acetate (3:1) as the eluent. Fractions containing the pure product were combined then concentrated in vacuo to a colorless oil; yield 54.2 g (66.7%). A portion (12.0 g) was transmitted to WRAIR on November 10, 1997 (Lot No. NJ26-63-2).

Anal.

| | <u>C</u> | <u>H</u> |
|------------------------------|----------|----------|
| Calc'd for $C_{24}H_{32}O_7$ | 66.65 | 7.46 |
| Found | 66.59 | 7.40 |

Spectral Data

Infrared (Nujol)

Major bands: ν 2950, 2920, 1715, 1605, 1450, 1430, 1370, 1270, 1220, 1185, 1168, 1150, 1130, 1095, 1020, 1002, 975, 945, 930, 865, 815, 740, 720 cm^{-1} .

Ultraviolet (Ethanol)

λ_{max} 236 nm (log ϵ 4.26).

Nuclear Magnetic Resonance ($CDCl_3$)

δ 8.01 (d, 2, aromatic H); 7.38 (d, 2, aromatic H); 5.45 (s, 1, H at C_{12}); 4.95 (d, 1, OCH_2); 4.91 (d, 1, H at C_{10}); 4.58 (d, 1, OCH_2); 3.91 (s, 3, OCH_3); 1.45 (s, 3, CH_3 at C_3); 0.96 (d, 3, CH_3 at C_6); 0.95 (d, 3, CH_3 at C_9).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

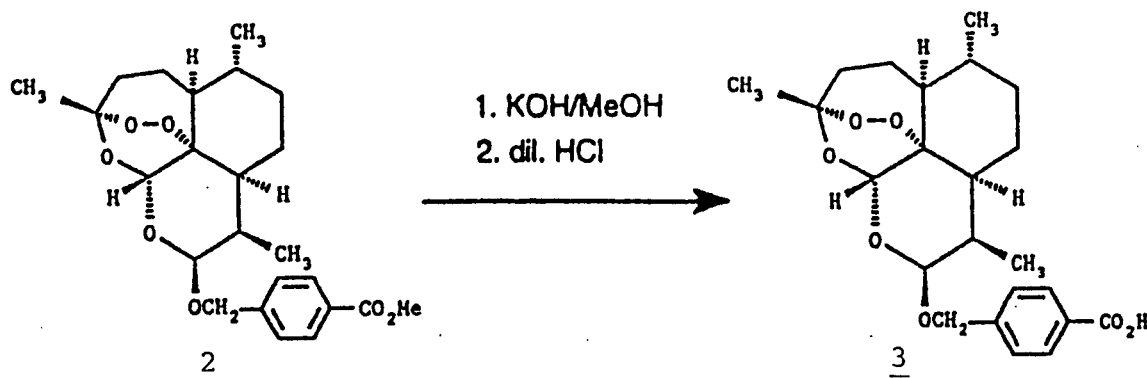
| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comments</u> |
|----|--------------------|-----------------|-----------------|
| 1. | Hexane-EtOAc (2:1) | 0.45 | Homogeneous |
| 2. | Hexane-EtOAc (3:1) | 0.32 | Homogeneous |

Source of Materials

- | | |
|--|----------------------------|
| 1. Dihydroartemisinin | Starks Associates, Inc. |
| 2. Ether | Fisher Chemical |
| 3. Methyl 4-hydroxymethyl-benzoate | Aldrich Chemical Co., Inc. |
| 4. $\text{BF}_3 \cdot \text{Et}_2\text{O}$ | Aldrich Chemical Co., Inc. |
| 5. NaHCO_3 | Aldrich Chemical Co., Inc. |
| 6. Distilled water | Mayer Bros. |
| 7. Na_2SO_4 | Aldrich Chemical Co., Inc. |
| 8. Silica Gel | E.M. Science |
| 9. Hexane | J.T. Baker Chemical Co. |
| 10. Ethyl acetate | J.T. Baker Chemical Co. |

13. Artelinic acid (3)

Reaction Sequence:



Experimental

Artelinic acid, methyl ester (2)

Please refer to the preceding synthesis, this report.

Artelinic acid (3)

A solution of KOH (28.5 g of 85% KOH, 0.432 mol) in methanol (550 mL) was added to the ester 2 (42.0 g, 0.092 mol). The reaction was stirred at room temperature for 7 days. The progress of the reaction was followed by TLC. The solution was acidified with acetic acid (67.5 mL) then concentrated in vacuo to a solid. The solid was partitioned between ether (1 L) and H₂O (1 L). The layers were separated and the organic portion washed with water (2 x 580 mL), dried (Na₂SO₄), then concentrated in vacuo to a white solid. This was dissolved in methanol (150 mL) and treated with decolorizing

carbon (1 g). To the filtered solution was added water (50 mL) and the material was allowed to crystallize. The white crystals obtained were filtered off and dried in vacuo at 40°C, yield 35.0 g (86.1%) mp 119-120°C; literature⁹ mp 142-145°C. A portion (10.0 g) was transmitted to WRAIR on November 10, 1997 (Lot No. NJ26-66-2).

Anal.

| | <u>C</u> | <u>H</u> | <u>O</u> |
|---|----------------|--------------|----------|
| Calc'd for $C_{23}H_{30}O_7 \cdot 0.5 H_2O$ | 64.62 | 7.31 | 28.07 |
| Found | 64.74 64.67 | 7.34 7.39 | 27.76 |

Spectral Data

Infrared (Nujol)

Major bands: ν 3380, 2900, 2830, 1700, 1655, 1605, 1568, 1580, 1450, 1420, 1385, 1370, 1305, 1270, 1240, 1218, 1168, 1198, 1120, 1090, 1050, 1015, 1000, 965, 945, 930, 915, 860, 835, 815, 740 cm^{-1} .

Ultraviolet (Ethanol)

λ_{max} 234 nm ($\log \epsilon$ 4.20).

Nuclear Magnetic Resonance ($CDCl_3$)

δ 8.09 (d, 2, H's at C_2 and C_6); 7.42 (d, 2, H's at C_3 and C_5); 5.46 (s, 1, H at C_{12}); 4.98 (d, 1, H at C_{10}); 4.93 (d, 1, CH_2); 4.60 (d, 1, CH_2); 2.71 (m, 1, H at C_9); 1.46 (s, 3, CH_3 at C_3); 0.98 (d, 3, CH_3 at C_6); 0.95 (d, 3, CH_3 at C_9).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comments</u> |
|----|---|-----------------|-----------------|
| 1. | Hexane-EtOAc (1:1) | 0.09 | Homogeneous |
| 2. | CH ₂ Cl ₂ -CH ₃ OH (9:1) | 0.36 | Homogeneous |

Source of Materials

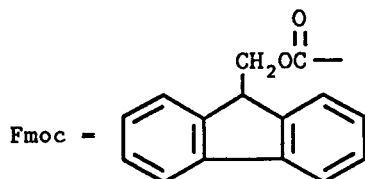
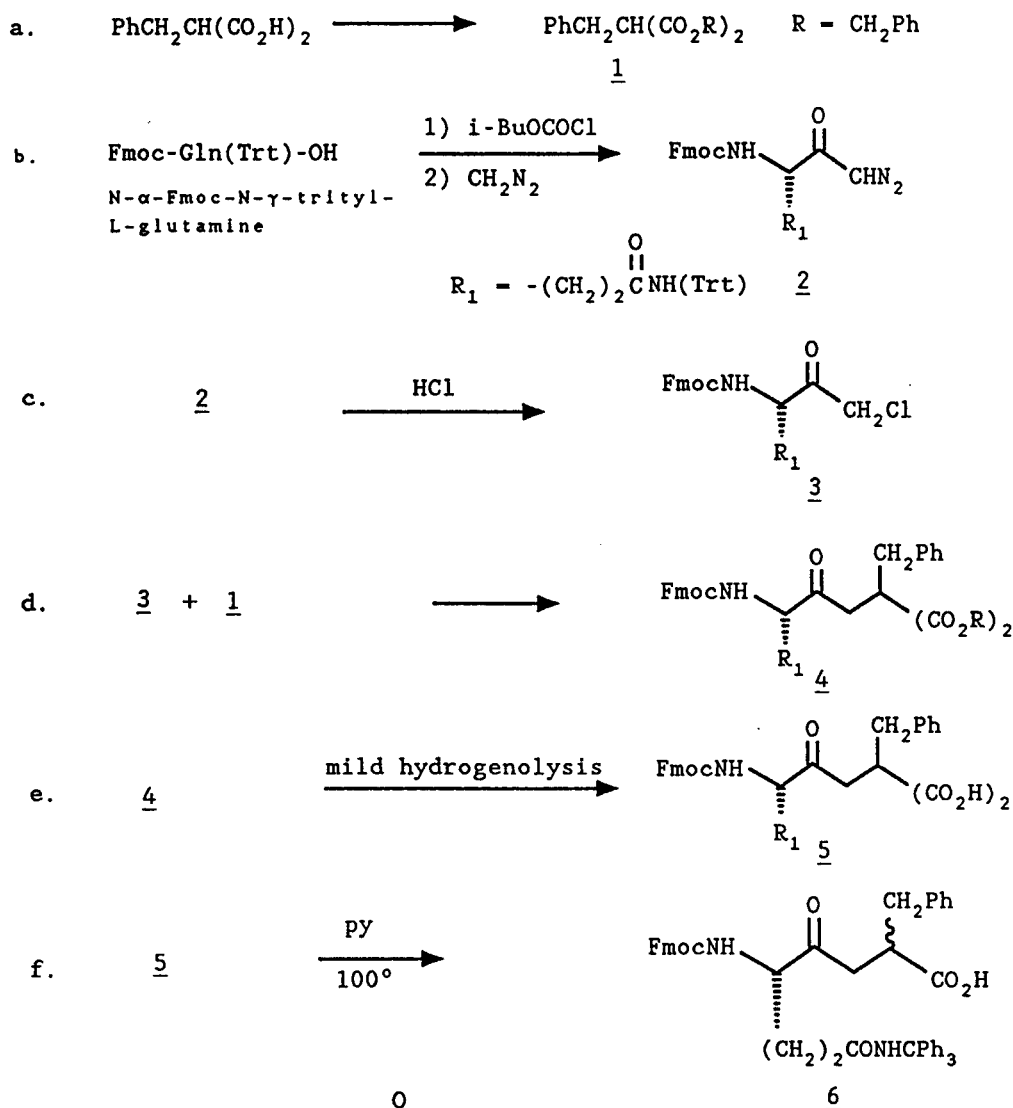
| | | |
|----|---------------------------------|----------------------------|
| 1. | Artelinic acid, methyl ester | Starks Associates, Inc. |
| 2. | KOH | Aldrich Chemical Co., Inc. |
| 3. | MeOH | J.T. Baker Chemical Co. |
| 4. | Acetic acid | J.T. Baker Chemical Co. |
| 5. | Ether | Fisher Chemical |
| 6. | Distilled water | Mayer Bros. |
| 7. | Na ₂ SO ₄ | J.T. Baker Chemical Co. |

B. Chemical Defense Related Compounds and Intermediates

14. Fmoc-L-Gln(Trt)ψ(COCH₂)-D,L-Phe (6)

The target compound 6 was prepared by the following reaction sequence. A portion (188 mg) was transmitted to WRAIR on January 7, 1997 (Lot No. NJ22-88-1).

Reaction Sequence:



Experimental¹⁰⁻¹⁵

Dibenzyl benzylmalonate (1)

Benzylmalonic acid (1.09 g, 6.7 mmol) was dissolved in MeOH (20 mL) and H₂O (2 mL), then titrated to pH7 (pH paper) with a 20% aqueous solution of Cs₂CO₃. The mixture was evaporated to dryness and the residue was evaporated from DMF (2 x 25 mL). The white solid obtained was stirred with benzyl bromide (1.8 mL, 15.1 mmol) in DMF (15 mL) for 20 h. The mixture was diluted with EtOAc (100 mL), washed with sat. NaHCO₃ (2 x 100 mL) and H₂O (100 mL), dried (MgSO₄), filtered and concentrated to give a residue (3 g) which was purified by column chromatography (60 g SiO₂, 1:9 EtOAc:hexanes) to give 1.1 g (44%) of pure (1).

Spectral Data:

Nuclear Magnetic Resonance (CDCl₃)

δ 7.30 - 7.10 (m, 15H, Ar); 5.09 (s, 4H, (CO₂CH₂Ph)₂); 3.76 (t, 1H, PhCH₂CH-); 3.24 (d, 2H, PhCH₂CH-).

Fmoc-L-Gln(Trt)-CH₂Cl (3)

4-Methylmorpholine (720 μ L, 6.55 mmol) and isobutyl chloroformate (850 μ L, 6.55 mmol) were added to a cooled solution (0 - 5°C) of N- α -Fmoc-N- γ -trityl-L-glutamine (4.0 g, 6.55 mmol) in 1 : 1 Et₂O:THF (120 mL). The mixture was stirred at 0-5°C for 1.0 h, filtered with suction and then added to an ethereal solution of diazomethane (alcohol-free, prepared from 5 g of Diazald, note 1) at 0-5°C. The reaction mixture was allowed to stand at 0-5°C for 2 h. The excess diazomethane was removed by purging the reaction mixture with argon while warming to 30-35°C (warm H₂O bath). The reaction

mixture was cooled to 0 - 5°C and a solution of 1.0M HCl in Et₂O (7.2 mL) was added dropwise. After 10 min at 0-5°C, sat. NaHCO₃ (100 mL) was added and the mixture was diluted with EtOAc (150 mL). The organic layer was separated, washed with H₂O (100 mL), dried (Na₂SO₄), filtered and concentrated to a residue, which was triturated with hexanes (200 mL) for 18 h. The suspension was suction filtered and the filter cake was dried in vacuo to give (3) as a white solid (2.4 g, 57%), mp 188-190°C.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.76-7.14 (m, 23H, Ar); 6.80 (s, 1H, -CONHTrt); 5.70 (d, 1H, Fmoc-NH-CH-); 4.52-4.3H (m, 3H, -COCH₂Cl and -N-CH-CO-); 4.21-4.08 (m, 3H, Fmoc CH and CH₂); 2.40-2.32 (m, 2H, TrtNHCOCH₂-); 2.18-2.15 (m, 1H, TrtNHCOCH₂-CH-); 1.88-1.85 (m, 1H, TrtNHCOCH₂-CH-).

Infrared (Nujol)

Major bands: 3380, 3240, 1720, 1670, 1490, 1265, 1240, 1035, 730, 700 cm⁻¹.

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|---------------------|-----------------|--|
| EtOAc-hexanes (1:2) | 0.28 | single major spot with lower Rf impurities |

Benzyl 5(S)-[(fluorenylmethoxycarbonyl)amino]-2-benzyl-2-(benzyloxycarbonyl)-4-oxooctanedioic acid, 8-N-triphenylmethanamide (4)

To a stirring suspension of 60% NaH (100 mg, 2.40 mmol) in THF (30 mL) under argon was added a solution of dibenzyl benzylmalonate (1) (884 mg, 2.36 mmol) in THF (20 mL). The reaction mixture was stirred at room temperature for 0.5 h, to give a clear solution of the mono-sodium salt of (1).

A mixture of Fmoc-L-Gln(Trt)-CH₂Cl (3) (1.39 g, 2.16 mmol) and sodium iodide (324 mg, 2.16 mmol) in THF (50 mL) was stirred at room temperature for 15 min. and then added to the solution of the mono-sodium salt of (1). Stirring was continued for 1.0 h then the solvent was concentrated and the residue was dissolved in EtOAc (150 mL) and washed with H₂O (75 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated to give the crude product (2.6 g), which was purified by column chromatography (140 g SiO₂, 1:2 EtOAc: hexanes). The resulting white residue was dissolved in EtOAc (25 mL) and diluted with hexanes (150 mL) to give a suspension which was suction filtered. The solid was washed with hexanes (20 mL) and dried in vacuo to give 898 mg (42%) of pure (4). Additional reactions gave a total of 987 mg of pure (4).

Spectral Data

¹H Nuclear Magnetic Resonance

δ 7.75-6.82 (m, 39H, Ar and TrtNHCO-); 5.53 (d, 1H, J= 7.2 Hz, -CONHCH-); 5.07 (m, 4H, -CO₂CH₂Ph); 4.37 (d, 2H, J=6.7 Hz, Fmoc CH₂); 4.17 (m, 2H, Fmoc CH and N-CH-CO); 3.40 (m, 2H, PhCH₂-); 3.12-2.91 (dd, 2H, J=8.73, 18.8 Hz, -CO-CH₂-); 2.21 (m, 1H, TrtNHCOCH-); 2.16 (m, 1H, TrtNHCOCH-); 2.04 (m, 1H, TrtNHCOCH₂-CH-); 1.61 (m, 1H, TrtNHCOCH₂-CH-).

Mass Spectrum

Method of Ionization = Electrospray (positive)

Calc'd for $C_{64}H_{56}N_2O_8$ = 980

Found: 1003 $(M+Na)^+$, 1004 $(M+Na+H)^+$

Chromatography

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|---------------------|-----------------|----------------|
| 1. | EtOAc:hexanes (2:3) | 0.51 | Homogeneous |

5(S)-[(Fluorenylmethoxycarbonyl)amino]-2-benzyl-2-carboxy-4-oxooctanedioic acid, 8-N-triphenylmethylethylamide (5)

A solution of benzyl 5(S)-[(fluorenylmethoxycarbonyl)-amino]-2-benzyl-2-(benzyloxycarbonyl)-4-oxooctanedioic acid, 8-N-triphenylmethylethylamide (0.50 g, 0.510 mmol) in EtOAc (60 mL) was hydrogenated at 30 psi in the presence of 10% palladium on carbon (400 mg) for 3 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness to give 0.41 g (100%) of crude (5) as light yellow solid. This material was used, as is, without further purification, in the next reaction. Additional reactions gave a total of 0.80 g of crude (5).

Fmoc-L-Gln(Trt)Ψ(COCH₂)-D,L-Phe (6)

A solution of 5(S)-[(fluorenylmethoxycarbonyl)amino]-2-benzyl-2-carboxy-4-oxooctanedioic acid, 8-N-triphenylmethylamide (0.41 g, 0.51 mmol) in pyridine (15 mL) was purged with argon and then stirred and heated at 100°C for 0.5 h. After cooling to room temperature, it was diluted with EtOAc (100 mL) and washed with 0.5N HCl (3 x 100 mL), H₂O (100 mL), and brine (2 x 100 mL). The organic layer was dried (MgSO₄), filtered, and concentrated to give a yellow oil (0.5 g). The oil was dissolved in EtOAc (20 mL) and diluted with hexanes (150 mL) to give a suspension, which was centrifuged to give a solid. The solid was washed with hexanes (20 mL) then dried in vacuo to give the crude target (268 mg). Additional reactions gave a total of 408 mg of crude target. The crude target (408 mg) was purified by column chromatography (25 g SiO₂, 98:2 CH₂Cl₂:CH₃OH followed by 49:2 CH₂Cl₂:CH₃OH) to give an oily residue which was dissolved in EtOAc (15 mL) and diluted with hexanes (125 mL) to give a white suspension. The solid was isolated by centrifugation, washed with hexanes (20 mL), and dried in vacuo for 6 h at room temperature to give 200 mg (26%) of pure target (6); mp 109-111°C. A portion (188 mg) was transmitted to WRAIR on January 7, 1997 (Lot No. NJ22-88-1).

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> |
|--|----------|----------|----------|
| Calc'd for C ₄₉ H ₄₄ N ₂ O ₆ | 77.11 | 6.05 | 3.62 |
| • 0.12 hexane • 0.4 H ₂ O | | | |
| Found | 77.11 | 6.00 | 3.60 |
| | 77.04 | 6.01 | 3.59 |

Spectral Data

¹H Nuclear Magnetic Resonance (DMSO-d₆)

(Mixture of two diastereomers)

δ 12.30 (m, 1 H, -COOH); 8.60 (s, 1H, NH); 7.96-7.13 (m, 29H, Ar and NH); 4.38-4.30 (m, 2H, Fmoc CH₂); 4.27-4.23 (m, 1H, Fmoc CH); 4.02-3.93 (m, 1H, N-CH-CO); 3.35 (H₂O in DMSO); 2.91-2.67 (3m, 4H, -COCH₂- and PhCH₂-); 2.55 (DMSO); 2.46-2.28 (m, 3H, -CHCOOH and TrtNHCOCH₂-); 1.97-1.86 (2m, 1H, TrtNHCOCH₂-CH-); 1.63-1.53 (2m, 1H, TrtNHCOCH₂-CH-); 1.30 (hexanes); 0.90 (hexanes).

FT-Infrared (thin film on KBr)

Major bands: 3385, 3363, 2926, 2855, 1746, 1716, 1495, 1445, 1333, 1276, 1226, 1204, 1190, 1158, 1091, 1017, 956, 904, 848, 815, 740, 701, 638, 599, 447 cm⁻¹.

Chromatography

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|--|-----------------|----------------|
| 1. | CH ₂ Cl ₂ :CH ₃ OH (98:2) | 0.12 | Homogeneous |
| 2. | CH ₂ Cl ₂ :CH ₃ OH (19:1) | 0.25 | Homogeneous |

Source of Materials:

| | |
|--|----------------------------|
| 1. Benzylmalonic acid | Aldrich Chemical Co., Inc. |
| 2. Methanol | J.T. Baker Chemical Co. |
| 3. Cesium carbonate | Cerac, Inc. |
| 4. N,N-Dimethylformamide | Aldrich Chemical Co., Inc. |
| 5. Benzyl bromide | Aldrich Chemical Co., Inc. |
| 6. Ethyl acetate | J.T. Baker Chemical Co. |
| 7. Sodium hydrogencarbonate | J.T. Baker Chemical Co. |
| 8. Magnesium sulfate | J.T. Baker Chemical Co. |
| 9. Silica gel | E.M. Science |
| 10. Hexanes | J.T. Baker Chemical Co. |
| 11. 4-Methylmorpholine | Aldrich Chemical Co., Inc. |
| 12. Isobutyl chloroformate | Aldrich Chemical Co., Inc. |
| 13. N- α -Fmoc-N- γ -trityl-L-glutamine | Nova Biochem |
| 14. Ethyl ether | Fisher Scientific |
| 15. Tetrahydrofuran | Aldrich Chemical Co., Inc. |
| 16. Diazald | Aldrich Chemical Co., Inc. |
| 17. 2-(2-Ethoxyethoxy)-ethanol | Aldrich Chemical Co., Inc. |
| 18. Potassium hydroxide | Aldrich Chemical Co., Inc. |
| 19. Hydrogen chloride (1.0M solution in diethyl ether) | Aldrich Chemical Co., Inc. |
| 20. Sodium sulfate | Aldrich Chemical Co., Inc. |
| 21. Sodium hydride (60% dispersion in mineral oil) | Aldrich Chemical Co., Inc. |
| 22. Sodium iodide | J.T. Baker Chemical Co. |

- | | | |
|-----|-------------------------|----------------------------|
| 23. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |
| 24. | Pyridine | Aldrich Chemical Co., Inc. |
| 25. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 26. | Dichloromethane | EM Science |

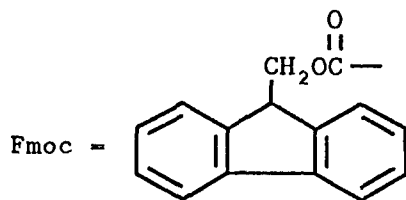
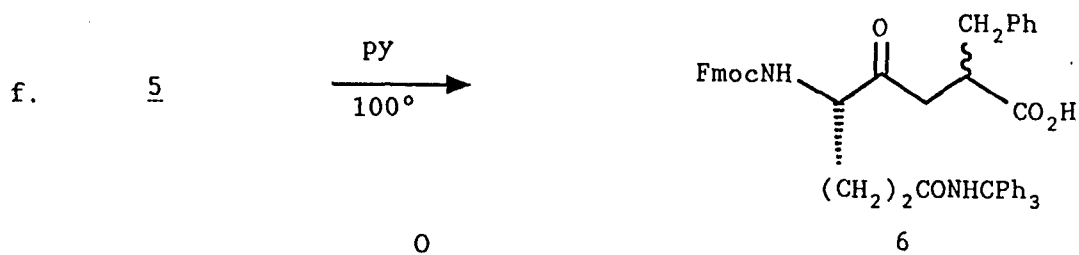
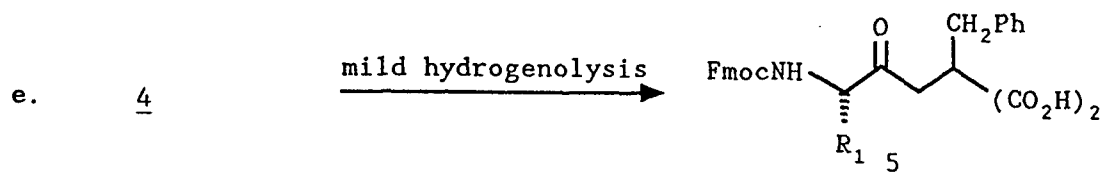
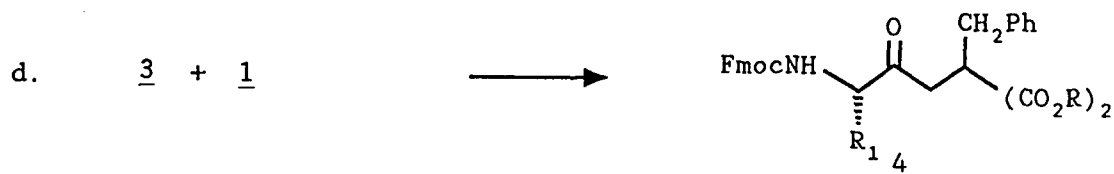
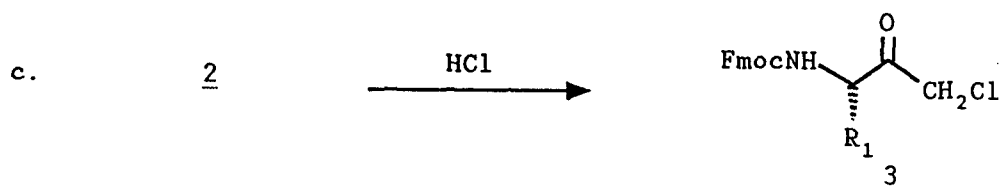
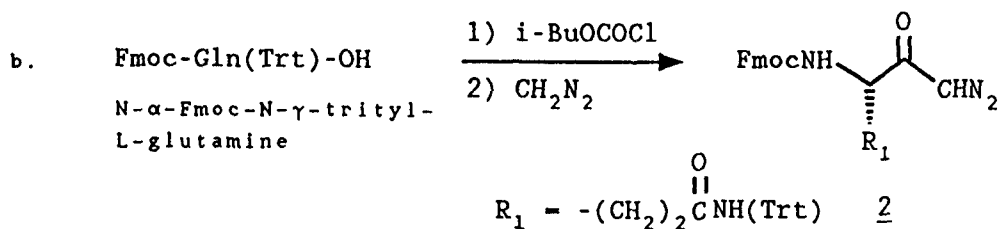
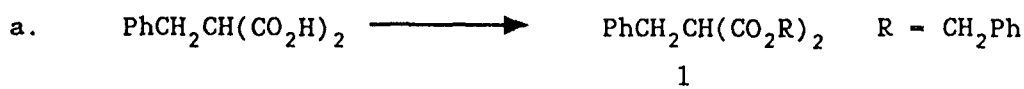
Note

1. Details for the preparation of alcohol-free diazomethane from Diazald are found in the Aldrich Chemical Co., Inc. Technical Information Bulletin Number AL-180, Section IV B (ii).

15. Fmoc-L-Gln(Trt)Ψ(COCH₂)-D,L-Phe (6)

The target compound 6 was prepared by the following reaction sequence. A portion (630 mg) was transmitted to WRAIR on April 4, 1997 (Lot No. NJ22-107-1).

Reaction Sequence:



Experimental¹⁰⁻¹⁵

Dibenzyl benzylmalonate (1)

Benzylmalonic acid (5.0 g, 31 mmol) was dissolved in MeOH (100 mL) and H₂O (10 mL), then titrated to pH7 (pH paper) with a 20% aqueous solution of Cs₂CO₃. The mixture was evaporated to dryness and the residue was evaporated from DMF (2 x 50 mL). The white solid obtained was stirred with benzyl bromide (8.3 mL, 70 mmol) in DMF (70 mL) for 20 h. The mixture was diluted with EtOAc (500 mL), washed with sat. NaHCO₃ (2 x 200 mL) and H₂O (2 x 200 mL), dried (MgSO₄), filtered and concentrated to give a residue (10.7 g) which was purified by column chromatography (200 g SiO₂, 1:9 EtOAc: hexanes) to give 3.6 g (31%) of pure (1).

Spectral Data:

Nuclear Magnetic Resonance (CDCl₃)

δ 7.30 - 7.10 (m, 15H, Ar); 5.09 (s, 4H, (CO₂CH₂Ph)₂); 3.76 (t, 1H, PhCH₂CH-); 3.24 (d, 2H, PhCH₂CH-).

Fmoc-L-Gln(Trt)-CH₂Cl (3)

4-Methylmorpholine (720 μL, 6.55 mmol) and isobutyl chloroformate (850 μL, 6.55 mmol) were added to a cooled solution (0 - 5°C) of N-α-Fmoc-N-γ-trityl-L-glutamine (4.0 g, 6.55 mmol) in THF (75 mL). The mixture was stirred at 0-5°C for 1.0 h, filtered with suction and then added to an ethereal solution of diazomethane (alcohol-free, prepared from 5 g of Diazald, note 1) at 0-5°C. The reaction mixture was allowed to stand at 0-5°C for 2 h. The excess diazomethane was

removed by purging the reaction mixture with argon while warming to 30-35°C (warm H₂O bath). The reaction mixture was cooled to 0 - 5°C and a solution of 1.0M HCl in Et₂O (7.2 mL) was added dropwise. After 10 min at 0-5°C, sat. NaHCO₃ (100 mL) was added and the mixture was diluted with EtOAc (150 mL). The organic layer was separated, washed with H₂O (100 mL), dried (Na₂SO₄), filtered and concentrated to a residue, which was triturated with hexanes (200 mL) for 18 h. The suspension was suction filtered and the filter cake was dried in vacuo to give (3) as a white solid (3.65 g, 87%), mp 188-190°C.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.76-7.14 (m, 23H, Ar); 6.80 (s, 1H, -CONHTrt); 5.70 (d, 1H, Fmoc-NH-CH-); 4.52-4.3H (m, 3H, -COCH₂Cl and -N-CH-CO-); 4.21-4.08 (m, 3H, Fmoc CH and CH₂); 2.40-2.32 (m, 2H, TrtNHCOCH₂-); 2.18-2.15 (m, 1H, TrtNHCOCH₂-CH-); 1.88-1.85 (m, 1H, TrtNHCOCH₂-CH-).

Infrared (Nujol)

Major bands: 3380, 3240, 1720, 1670, 1490, 1265, 1240, 1035, 730, 700 cm⁻¹.

Chromatography

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|---------------------|-----------------|--|
| EtOAc-hexanes (1:2) | 0.28 | single major spot with trace lower Rf impurities |

Benzyl 5(S)-[(fluorenylmethoxycarbonyl)amino]-2-benzyl-2-(benzyloxycarbonyl)-4-oxooctanedioic acid, 8-N-triphenylmethanamide (4)

To a stirring suspension of 60% NaH (242 mg, 6.05 mmol) in THF (75 mL) under argon was added a solution of dibenzyl benzylmalonate (1) (2.22 g, 5.93 mmol) in THF (50 mL). The reaction mixture was stirred at room temperature for 0.5 h, to give a clear solution of the mono-sodium salt of (1).

A mixture of Fmoc-L-Gln(Trt)-CH₂Cl (3) (3.50 g, 5.44 mmol) and sodium iodide (815 mg, 5.44 mmol) in THF (125 mL) was stirred at room temperature for 15 min. and then added to the solution of the mono-sodium salt of (1). Stirring was continued for 1.0 h then the solvent was concentrated and the residue was dissolved in EtOAc (300 mL) and washed with H₂O (150 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated to give the crude product (5.9 g), which was purified by column chromatography (300 g SiO₂, 2:3 EtOAc: hexanes). The resulting white residue was dissolved in EtOAc (50 mL) and diluted with hexanes (400 mL) to give a suspension which was suction filtered. The solid was washed with hexanes (50 mL) and dried in vacuo to give 2.2 g (41%) of pure (4).

Spectral Data

¹H Nuclear Magnetic Resonance

δ 7.75-6.82 (m, 29H, Ar and TrtNHCO-); 5.53 (d, 1H, J= 7.2 Hz, -CONHCH-); 5.07 (m, 4H, -CO₂CH₂Ph); 4.37 (d, 2H, J=6.7 Hz, Fmoc CH₂); 4.17 (m, 2H, Fmoc CH and N-CH-CO); 3.40 (m, 2H, PhCH₂-); 3.12-2.91 (dd, 2H, J=8.73, 18.8 Hz, -CO-CH₂-); 2.21 (m, 1H, TrtNHCOCH-); 2.16 (m, 1H, TrtNHCOCH-); 2.04 (m, 1H, TrtNHCOCH₂-CH-); 1.61 (m, 1H, TrtNHCOCH₂-CH-).

Chromatography

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|---------------------|-----------------|----------------|
| 1. | EtOAc:hexanes (2:3) | 0.51 | Homogeneous |

5(S)-[(Fluorenylmethoxycarbonyl)amino]-2-benzyl-2-carboxy-4-oxooctanedioic acid, 8-N-triphenylmethanamide (5)

A solution of benzyl 5(S)-[(fluorenylmethoxycarbonyl)amino]-2-benzyl-2-(benzyloxycarbonyl)-4-oxooctanedioic acid, 8-N-triphenylmethanamide (1.10 g, 1.12 mmol) in EtOAc (100 mL) was hydrogenated at 30 psi in the presence of 10% palladium on carbon (880 mg) for 3 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness to give 0.9 g (100%) of crude (5) as light yellow solid. This material was used, as is, without further purification, in the next reaction. Additional reactions gave a total of 1.64 g of crude (5).

Fmoc-L-Gln(Trt)ψ(COCH₂)-D,L-Phe (6)

A solution of 5(S)-[(fluorenylmethoxycarbonyl)amino]-2-benzyl-2-carboxy-4-oxooctanedioic acid, 8-N-triphenylmethanamide (0.9 g, 1.12 mmol) in pyridine (33 mL) was purged with argon and then stirred and heated at 100°C for 0.5 h. Cooled to room temperature, diluted with EtOAc (250 mL) and washed

with 0.5N HCl (3 x 150 mL) and brine (3 x 150 mL). The organic layer was dried (MgSO_4), filtered, and concentrated to give a yellow oil (1.4 g). The oil was dissolved in EtOAc (50 mL) and diluted with hexanes (250 mL) to give a suspension, which was centrifuged to give a solid. The solid was washed with hexanes (20 mL) then dried in vacuo to give the crude target (665 mg). Additional reactions gave a total of 1.2 g of crude target (6). The crude target (1.2 g) was purified by column chromatography (80 g SiO_2 , 98:2 CH_2Cl_2 : CH_3OH followed by 49:2 CH_2Cl_2 : CH_3OH) to give an oily residue which was dissolved in EtOAc (35 mL) and diluted with hexanes (350 mL) to give a white suspension. The solid was isolated by centrifugation, washed with hexanes (20 mL), and dried in vacuo for 6 h at room temperature to give 668 mg (43%) of pure target (6); mp 109-111°C. A portion (630 mg) was transmitted to WRAIR on April 4, 1997 (Lot No. NJ22-107-1).

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> |
|---|----------|----------|----------|
| Calc'd for $\text{C}_{49}\text{H}_{44}\text{N}_2\text{O}_6$ | 77.05 | 5.99 | 3.63 |
| • 0.04 EtOAc • 0.06 hexane • 0.35 H_2O | | | |
| Found | 77.04 | 6.00 | 3.67 |
| | 77.03 | 6.05 | 3.62 |

Spectral Data

¹H Nuclear Magnetic Resonance (DMSO-d₆)

(Mixture of two diastereomers)

δ 8.60 (d, 1H, J= 3.6 Hz, NH); 7.96-7.13 (m, 29H, Ar and NH); 4.38-4.30 (m, 2H, Fmoc CH₂); 4.27-4.23 (m, 1H, Fmoc CH); 4.08 (EtOAc); 4.02-3.93 (m, 1H, N-CH-CO); 3.35 (H₂O in DMSO); 3.02-2.66 (3m, 4H, -COCH₂- and PhCH₂-); 2.55 (DMSO); 2.47-2.29 (m, 3H, -CHCOOH and TrtNHCOCH₂-); 2.04 (EtOAc); 1.98-1.85 (2m, 1H, TrtNHCOCH₂-CH-); 1.66-1.51 (2m, 1H, TrtNHCOCH₂-CH-); 1.30 (hexane); 1.23 (EtOAc); 0.90 (hexane).

FT-Infrared (thin film on KBr)

Major bands: 3229, 3062, 3028, 2928, 1710, 1509, 1495, 1448, 1245, 1085, 1035, 1003, 909, 844, 759, 736, 700, 638, 621 cm⁻¹.

Chromatography

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|---|-----------------|----------------|
| 1. CH ₂ Cl ₂ :CH ₃ OH (19:1) | 0.19 | Homogeneous |
| 2. CH ₂ Cl ₂ :CH ₃ OH (10:1) | 0.43 | Homogeneous |

Source of Materials:

| | |
|--|----------------------------|
| 1. Benzylmalonic acid | Aldrich Chemical Co., Inc. |
| 2. Methanol | J.T. Baker Chemical Co. |
| 3. Cesium carbonate | Cerac, Inc. |
| 4. N,N-Dimethylformamide | Aldrich Chemical Co., Inc. |
| 5. Benzyl bromide | Aldrich Chemical Co., Inc. |
| 6. Ethyl acetate | J.T. Baker Chemical Co. |
| 7. Sodium hydrogencarbonate | J.T. Baker Chemical Co. |
| 8. Magnesium sulfate | J.T. Baker Chemical Co. |
| 9. Silica gel | E.M. Science |
| 10. Hexanes | J.T. Baker Chemical Co. |
| 11. 4-Methylmorpholine | Aldrich Chemical Co., Inc. |
| 12. Isobutyl chloroformate | Aldrich Chemical Co., Inc. |
| 13. N- α -Fmoc-N- γ -trityl-L-glutamine | Nova Biochem |
| 14. Ethyl ether | Fisher Scientific |
| 15. Tetrahydrofuran | Aldrich Chemical Co., Inc. |
| 16. Diazald | Aldrich Chemical Co., Inc. |
| 17. 2-(2-Ethoxyethoxy)-ethanol | Aldrich Chemical Co., Inc. |
| 18. Potassium hydroxide | Aldrich Chemical Co., Inc. |
| 19. Hydrogen chloride (1.0M solution in diethyl ether) | Aldrich Chemical Co., Inc. |
| 20. Sodium sulfate | Aldrich Chemical Co., Inc. |
| 21. Sodium hydride (60% dispersion in mineral oil) | Aldrich Chemical Co., Inc. |
| 22. Sodium iodide | J.T. Baker Chemical Co. |

- | | | |
|-----|-------------------------|----------------------------|
| 23. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |
| 24. | Pyridine | Aldrich Chemical Co., Inc. |
| 25. | Hydrochloric acid | J.T. Baker Chemical Co. |
| 26. | Dichloromethane | EM Science |

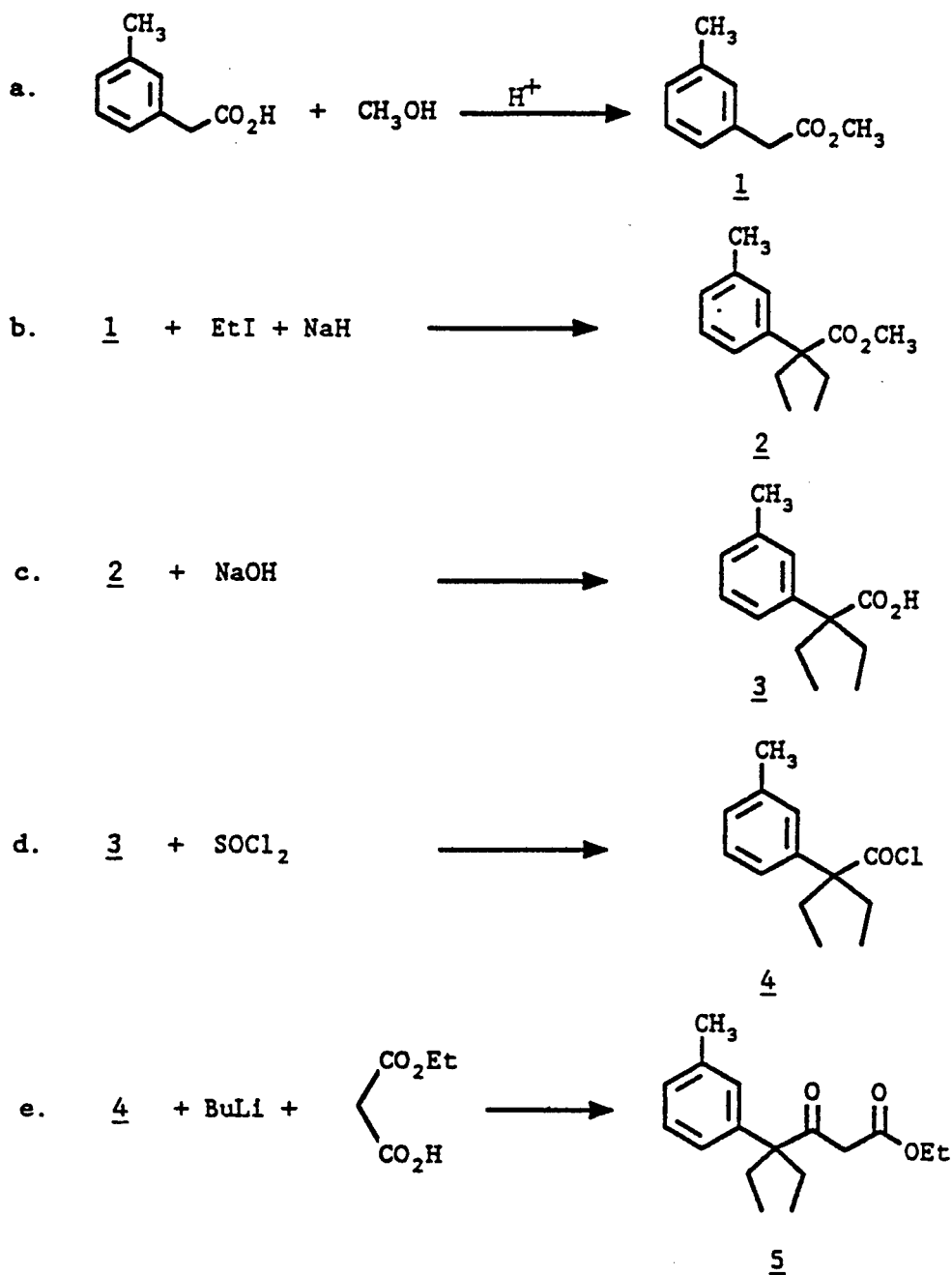
Note

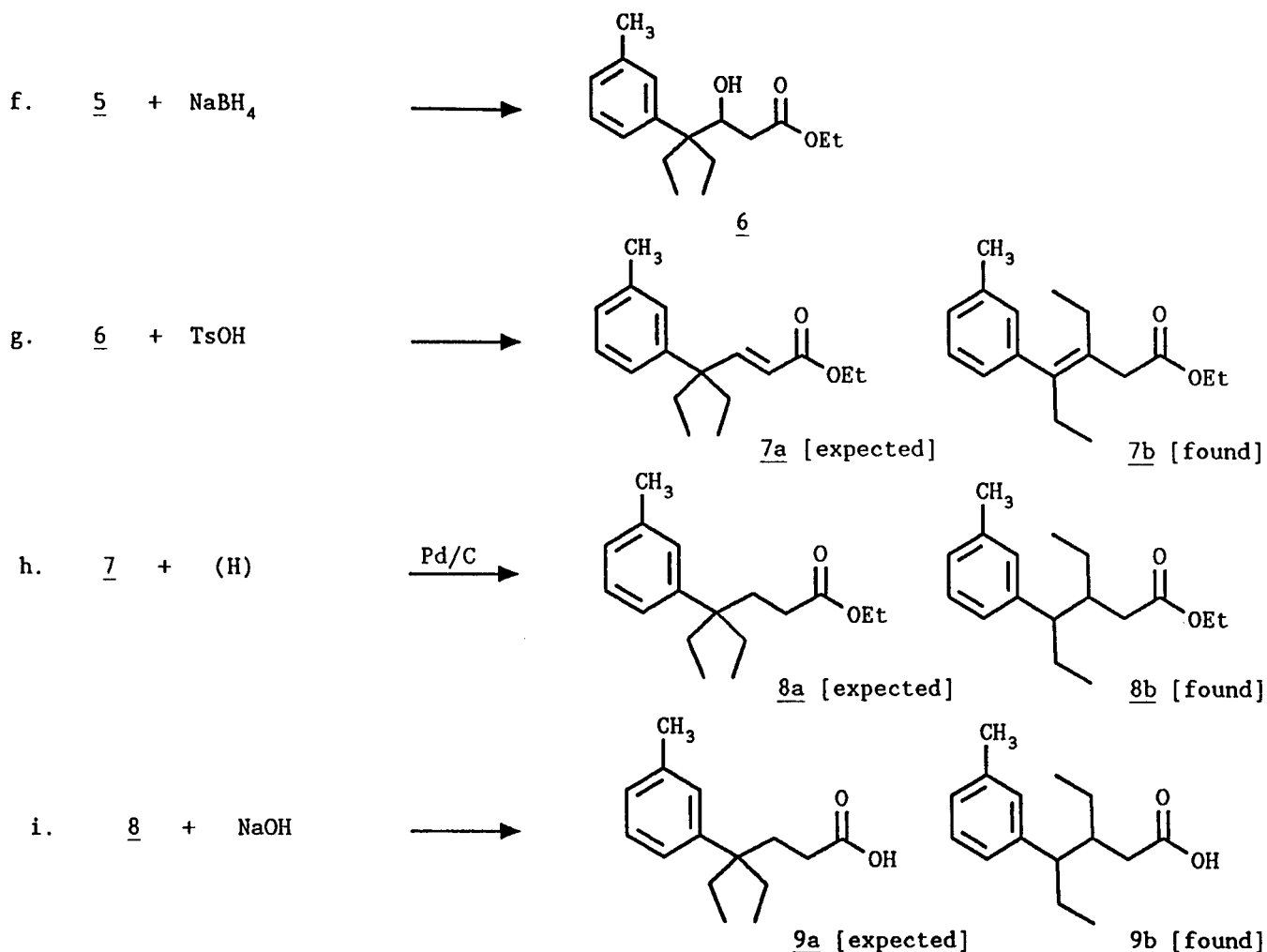
1. Details for the preparation of alcohol-free diazomethane from Diazald are found in the Aldrich Chemical Co., Inc. Technical Information Bulletin Number AL-180, Section IV B (ii).

16. Hexanoic acid, 3-ethyl-4-(3-methylphenyl)- (9b)

The proposed reaction sequence for a model compound 9a is shown below. Instead of the expected material 9a, however, a different product was isolated, which was identified as 9b. The synthesis was a model for the preparation of ligand 09E.

Reaction Sequence:





Experimental

Acetic acid, 3-methylphenyl- methyl ester (1)

m-Tolylacetic acid (25.0 g, 0.166 mol) was dissolved in methanol (25.0 g, 0.78 mol), containing 1.0 g of conc. H₂SO₄. The mixture was heated under reflux for 16 h, cooled, and concentrated in vacuo to an oil. The oil was dissolved in CH₂Cl₂ (200 mL) and mixed with H₂O (100 mL). The stirred mixture was neutralized with NaHCO₃, and the layers were separated. The organic layer was washed with H₂O (2 x 100 mL), dried over MgSO₄ and concentrated in vacuo to an oil (24.8 g) (90.8% yield). The material was suitable for further transformation. Additional product (59.4 g) was obtained in a similar fashion.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.15-6.90 (m, 4, aromatic H); 3.62 (s, 3, -CO₂CH₃); 3.50 (s, 2, CH₂); 2.28 (s, 3, ArCH₃).

Butanoic acid, 2-ethyl-2-(3-methylphenyl)- methyl ester (2)

m-Tolylacetic acid, methyl ester 1 (23.0 g, 0.14 mol) was dissolved in dry THF (200 mL) and cooled below 0°C under argon. NaH (60% min. oil dispersion (20.7 g, 0.52 mol)) was added to the solution followed by iodoethane (79.1 g, 0.51 mol) dissolved in THF (160 mL). The reaction was warmed slowly to room temperature over ~30 min. It was stirred for 3.5 h at this temperature and then heated under reflux for 2 h. After stirring at room temperature for 60 h, the reaction mixture was cooled to 4°C in an ice bath and H₂O (50 mL) was added dropwise over ~20 min. keeping the temperature below 15°C. The quenched reaction mixture was concentrated in vacuo to an oil (133.0 g) . This was dissolved in EtOAc (1 L) and washed with H₂O (2 x 800 mL). The combined aqueous portions were extracted with EtOAc (2 x 250 mL). Organic extracts were combined and washed with brine (500 ml), dried over MgSO₄, and concentrated to 35.2 g of a yellow oil. This was chromatographed on a 725 g silica gel column using hexane/EtOAc (9:1) as the eluent to give 24.8 g of product in 80.6% yield. This material was suitable for further transformation. Additional product (45.8 g) was obtained from a similar reaction.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.25-7.02 (m, 4, aromatic H); 3.63 (s, 3, -CO₂CH₃); 2.32 (s, 3, ArCH₃); 2.02 (m, 4, CH₂); 0.71 (t, 6, CH₃).

Butanoic acid, 2-ethyl-2-(3-methylphenyl)- (3)

A mixture of 2 (10.0 g, 45.4 mmol), NaOH (10.0 g, 250 mmol) and EtOH/H₂O (45 mL/9 mL) was heated with stirring under reflux for 4.5 h and then stirred overnight at room temperature. It was poured into cold H₂O (760 mL), acidified with diluted HCl and extracted with CH₂Cl₂ (2 x 150 mL). The organic extract was washed with H₂O (2 x 150 mL) then concentrated to an oil. This was suspended in H₂O (600 mL) then basified with 6 M NaOH. The organic extract was washed with H₂O (2 x 150 mL), dried over MgSO₄, and concentrated to obtain 8.6 g, (91.8%) of 3 as a white solid suitable for further transformation, mp 100-102°C. Additional material (6.4 g) was obtained in a similar fashion.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 12.24 (s, 1, COOH); 7.25-7.09 (m, 4, aromatic H); 2.34 (s, 3, ArCH₃); 1.97 (m, 4, CH₂); 0.72 (t, 6, CH₃).

Butanoyl chloride, 2-ethyl-2-(3-methylphenyl)- (4)

Compound 3 (4.4 g, 21.3 mmol) was dissolved in SOCl_2 (10 mL, 137 mmol) then heated under reflux for 3 h. Excess SOCl_2 was removed by vacuum distillation. The acid chloride (4.78 g, assumed 100% conversion) was used in the next step without characterization.

Hexanoic acid, 4-ethyl-4-(3-methylphenyl)-3-oxo-ethyl ester (5)

Monoethyl malonate (7.0 g, 52.98 mmol) was dissolved in dry THF (60 mL) and 5 mg of 2,2'-bipyridyl was added. The solution was cooled to approximately -70°C under argon, and a solution of 1.6 M of butyllithium in hexane was added dropwise while the temperature was allowed to rise to -5° . Sufficient BuLi was added (approx. 65 mL) until a pink color persisted for several min. The reaction mixture was recooled to -70°C and a solution of 2,2-diethyl-(3-methylphenyl)acetyl chloride 4 (4.28 g, 213 mol) in THF (10 mL) was added dropwise. The reaction solution was stirred overnight at room temperature under argon, then poured into Et_2O (400 mL) and 1N HCl (100 mL). The layers were separated, and the organic phase was washed with sat. NaHCO_3 solution (2 x 100 mL), followed by H_2O (2 x 100 mL). It was dried over MgSO_4 and after removal of solvent under reduced pressure, 6.7 g crude product was obtained as an oil. This was chromatographed on 1 kg silica gel column with hexane/ EtOAc (2:1) as eluent to obtain 2.39 g, (40.5%) of 5 as a brown oil. Additional product (1.07 g) was obtained from a scouting run.

Anal.

| | <u>C</u> | <u>H</u> |
|---|----------|----------|
| Calc'd for $\text{C}_{17}\text{H}_{24}\text{O}_3$ | 73.88 | 8.74 |
| Found | 73.77 | 8.77 |

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.26-6.99 (m, 4, aromatic - H's); 4.10 (q, 2, CO₂CH₂) 3.17 (s, 2, CH₂ - CO₂C₂H₅); 2.34 (s, 3, Ar-CH₃); 1.99 (m, 4, 2 x CH₂); 1.22 (t, 3, CO₂- CH₂ CH₃); 0.70 (t, 6, 2 x CH₂CH₃).

Infrared (Neat)

2975, 2870, 1745, 1710, 1605, 1460, 1410, 1385, 1370, 1315, 1260, 1140, 1030, 775 cm⁻¹.

Hexanoic acid, 4-ethyl-3-hydroxy-4-(3-methylphenyl)-ethyl ester (6)

4-Ethyl-4-(3-methylphenyl)-3-oxohexanoic acid, ethyl ester (0.99 g 3.58 mmol) was dissolved in CH₃OH (25 mL) and cooled to 2°C under argon. Solid NaBH₄ (1.01 g, 26.7 mmol) was added portionwise over 30 min. while maintaining the reaction temperature below 5°C. The reaction mixture was warmed to room temperature in a water bath and stirred for 1 h. It was quenched by dropwise addition of a mixture of acetic acid (1.7 mL) and CH₃OH (8.6 mL). The solvent was removed and the residue was suspended in CH₂Cl₂ (230 mL) and sat. NaHCO₃ (230 mL). The aqueous phase was extracted with CH₂Cl₂ (30 mL). The organic extracts were combined, washed with brine (2 x 40 mL) and dried over MgSO₄. Removal of the solvent in vacuo resulted in 0.84 g of oil. This was purified on a 120 g silica gel column using hexane/EtOAc 5:1 as the eluent to obtain 0.31 g of product as an oil. The high field NMR spectrum was consistent with the

structure of 6 and TLC of the product showed one major spot (Rf= 0.43; hexane:EtOAc (4:1)).

3-Hexenoic acid, 3-ethyl-4-(3-methylphenyl)-ethyl ester (7b)

A mixture of 4-ethyl-3-hydroxy-4-(3-methylphenyl)hexanoic acid ethyl ester (0.31 g, 1.1 mmol) and *p*-toluenesulfonic acid (59 mg, 0.31 mmol) in dry toluene (30 ml), under argon, was heated to reflux (112°C) using a Dean-Stark trap to remove water. After heating overnight (~20 h) the mixture was cooled and concentrated to 0.34 g oily solid. The product was purified on a 16 g column of silica gel eluted with hexane:EtOAc (9:1). Fractions containing the desired product were concentrated to yield 0.15 g oil (0.58 mmol, 51.7%).

Hexanoic acid, 3-ethyl-4-(3-methylphenyl)-ethyl ester (8b)

A mixture of 3-ethyl-4-(3-methylphenyl)-3-hexenoic acid ethyl ester (0.15 g, 0.58 mmol) and 5% Pd on carbon (25 mg) in ethanol (15 mL) was shaken for 34 h. on the hydrogenation apparatus (36 psi. H₂). The mixture was filtered to remove the catalyst then concentrated to 0.13 g oil. The oil was purified on a 20 g column of silica gel eluted with hexanes:CH₂Cl₂ (1:1); fractions containing the product were concentrated to 0.108 g oil. TLC of the purified product showed one major spot with two minor impurities; the NMR spectrum was consistent with the structure.

Hexanoic acid, 3-ethyl-4-(3-methylphenyl)- (9b)

A mixture of 3-ethyl-4-(3-methylphenyl)hexanoic acid ethyl ester (50 mg, 0.19 mmol) and NaOH (800 mg, 20 mmol) in EtOH/H₂O (4 mL/0.75 mL) was heated at reflux (84°C) for 2 h. After cooling, the reaction mixture was mixed with water (45 mL) and acidified with 6M HCl (~3.3 mL). After stirring 10 min. the pH was rechecked and an additional 0.15 mL acid was added. The aqueous suspension was extracted with CH₂Cl₂ (3 x 10 mL) and the combined extracts washed with water (2 x 15 mL) then concentrated to an oil. The oil was suspended in water (3 mL) and basified with 6M NaOH (~35 µL). The aqueous mixture was washed with CH₂Cl₂ (4 x 1 mL) then acidified with 6M HCl (25-30 µL) and extracted with CH₂Cl₂ (4 x 1 mL). The combined organic portion was reduced in volume (under a stream of argon) to 2.5 mL, washed with water (2 x 1.5 mL), dried over Na₂SO₄ and concentrated to 32.0 mg clear colorless oil. Infrared and high field NMR spectra were both consistent with the structure of (9b) and the molecular weight was confirmed by the mass spectrum; TLC (hexane:EtOAc (2:1)) of the product showed a single major spot and two very faint impurities. A portion (25 mg) was transmitted to WRAIR on June 25, 1997 (Lot No. NJ23-71-1).

Spectral Data

Infrared (Neat)

3020, 2950, 2920, 2860, 1710, 1600, 1485, 1455,
1410, 1380, 1280, 1170, 1090, 775 cm⁻¹.

Nuclear Magnetic Resonance (CDCl₃)

δ 7.16-6.94 (m, 4, aromatic - H's); 3.11 (m, 1, CH-Ø); 2.73 (d of d, J= 15.6 Hz, 5.1 Hz, CHCO); 2.60 (d of d, J= 15.6 Hz, 5.1 Hz, CHCO); 2.39 (s, 3, CH₃); 1.40 (q, 2, J= 7.4 Hz, CH₂); 1.30 (q, 2, J= 7.4 Hz, CH₂); 0.88 (t, 3, J= 7.4 Hz, CH₃); 0.81 (t, 3, J= 7.4 Hz, CH₃).

Mass Spectrometry (Acq method)

m/z 234 (m, abund 34); 235 (m+1, abund 10)

Source of Materials

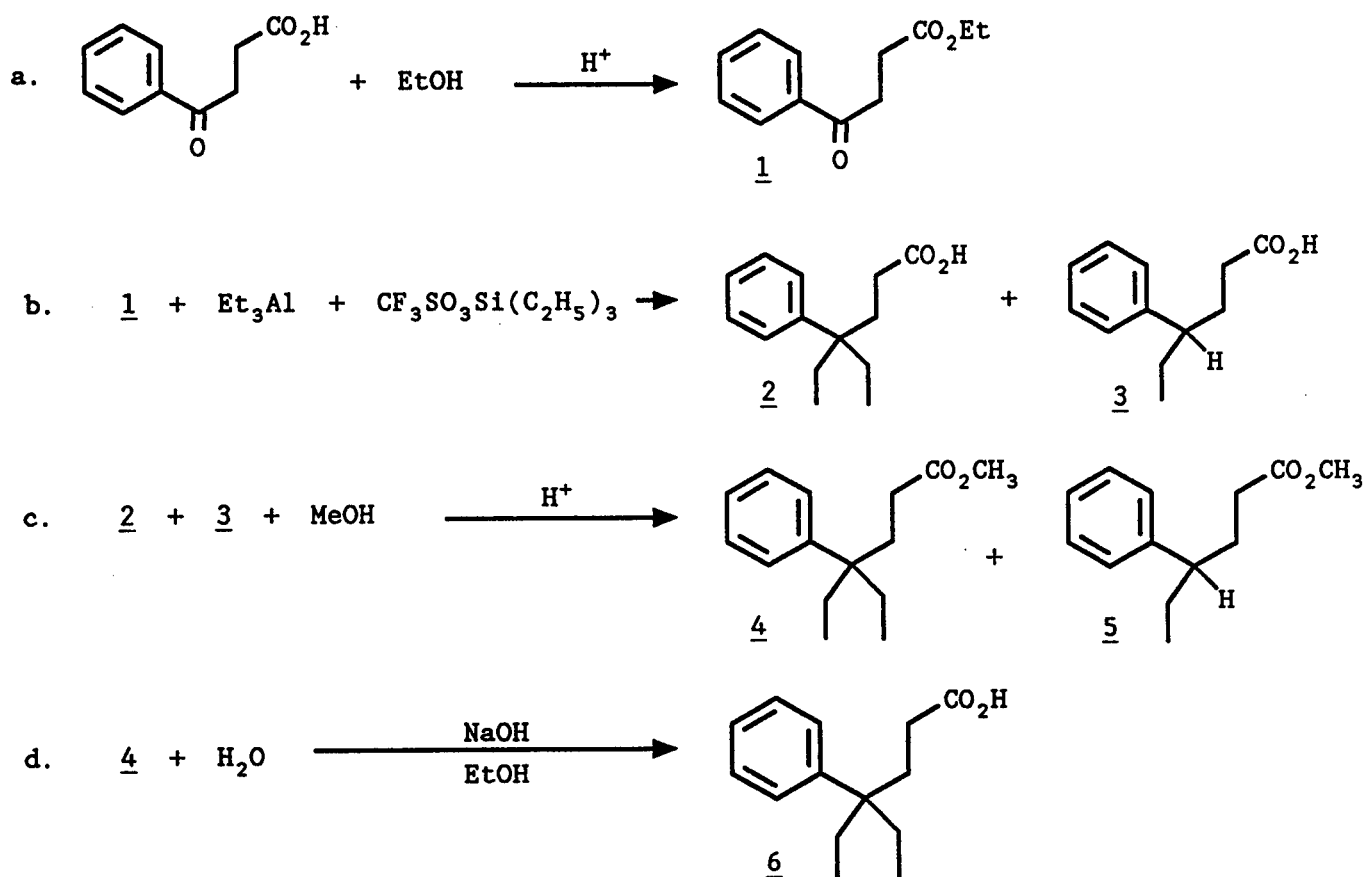
| | |
|--|----------------------------|
| 1. <i>m</i> -Tolylacetic acid | Aldrich Chemical Co., Inc. |
| 2. Methanol | J.T. Baker Chemical Co. |
| 3. H ₂ SO ₄ | J.T. Baker Chemical Co. |
| 4. CH ₂ Cl ₂ | J.T. Baker Chemical Co.. |
| 5. NaHCO ₃ | Aldrich Chemical Co., Inc. |
| 6. Methyl 3-methylphenyl- acetate | Starks Associates, Inc. |
| 7. THF | Aldrich Chemical Co., Inc. |
| 8. NaH | Aldrich Chemical Co., Inc. |
| 9. CH ₃ I | Aldrich Chemical Co., Inc. |
| 10. EtOAc | J.T. Baker Chemical Co. |
| 11. Silica gel | EM Laboratories |
| 12. Methyl 2-ethyl-2- (3-methylphenyl)butanoate | Starks Associates, Inc. |
| 13. NaOH | Aldrich Chemical Co., Inc. |
| 14. EtOH | US Industries |
| 15. 2-Ethyl-2-(3-methylphenyl)- butanoic acid | Starks Associates, Inc. |

| | | |
|-----|---|----------------------------|
| 16. | SOCl_2 | Aldrich Chemical Co., Inc. |
| 17. | 2-Ethyl-2-(3-methylphenyl)- butanoyl chloride | Starks Associates, Inc. |
| 18. | Monoethyl malonate | Aldrich Chemical Co., Inc. |
| 19. | 2,2'-Bipyridyl | Aldrich Chemical Co., Inc. |
| 20. | <i>n</i> -Butyllithium (1.6 M in hexane) | Aldrich Chemical Co., Inc. |
| 21. | Et_2O | Fisher Scientific |
| 22. | Ethyl 4-ethyl-4-(3-methyl- phenyl)-3-oxohexanoate | Starks Associates, Inc. |
| 23. | NaBH_4 | Aldrich Chemical Co., Inc. |
| 24. | Ethyl 4-ethyl-3-hydroxy- 4-(3-methylphenyl)hexanoate | Starks Associates, Inc. |
| 25. | <i>p</i> -Toluenesulfonic acid | Starks Associates, Inc. |
| 26. | Toluene | J.T. Baker Chemical Co. |
| 27. | Ethyl 3-ethyl-4-(3-methyl- phenyl)-3-hexenoate | Starks Associates, Inc. |
| 28. | 5% Pd/C | Aldrich Chemical Co., Inc. |
| 29. | Ethyl 3-ethyl-4-(3-methyl- phenyl)hexanoate | Starks Associates, Inc. |

17. Hexanoic acid, 4-ethyl-4-phenyl- (6)

The target compound 6 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

Propionic acid, 3-benzoyl- ethyl ester (1)

To a solution of 3-benzoylpropionic acid (5 g, 28 mmol) in EtOH (10 mL) was added sulfuric acid (0.2 g) and the solution was refluxed for 6 h, cooled, then concentrated in vacuo to an oil. The oil was dissolved in CH₂Cl₂ (50 mL), and the solution was washed with saturated NaHCO₃ (2 x 25 mL), H₂O (25 mL), dried (MgSO₄), then concentrated in vacuo to an oil; yield, 5.3 g (97.4%). The material was suitable for further transformation.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 1.18 (t, 3, CH₃); 2.67 (t, 2, CH₂CH₂CO₂Et); 3.23 (t, 2, CH₂CH₂CO₂Et); 4.07 (q, 2, CH₂CH₃); 7.40 (m, 3, aromatic H); 7.83 (m, 2, aromatic H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf (3-benzoyl- propionic acid)</u> | <u>Rf (1)</u> |
|----|---|---|---------------|
| 1. | CH ₂ Cl ₂ -MeOH (4:1) | 0.56 | 0.74 |
| 2. | Hexane-EtOAc (1:1) | 0.14 | 0.73 |

Butanoic acid, 4,4-diethyl-4-phenyl- (2) and
butanoic acid, 4-ethyl-4-phenyl- (3)

To a cooled (0°C) solution of 1 (3.5 g, 18.0 mmol) in CH₂Cl₂ (25 mL) was added dropwise triethylaluminum (36 mL of 1 M solution), followed by dropwise addition of triethylsilyl trifluoromethanesulfonate (8 mL). The mixture was stirred at 0°C for 1 h, at RT for 16 h then poured into a mixture of ice/ether (200 mL) and acidified with H₃PO₄. The mixture was extracted with ether (3 x 100 mL), dried (MgSO₄), then concentrated in vacuo (6.4 g). The material was purified by chromatography on a column of SiO₂ (100 g) using CH₂Cl₂-MeOH (4:1) as the eluent. Fractions containing the product 2 & 3 were combined then concentrated in vacuo; yield 2.4 g.

Butanoic acid, 4,4-diethyl-4-phenyl- methyl ester (4)

The mixture of 2 & 3 (2.4 g), was dissolved in CH₃OH (25 mL) and 1 drop of H₂SO₄ was added. The mixture was refluxed for 3 h, cooled then concentrated in vacuo. The residue was dissolved in CH₂Cl₂ (120 mL), washed with sat. NaHCO₃ solution (60 mL), H₂O (2 x 60 mL), dried (MgSO₄) then concentrated in vacuo to an oil (1.7 g). The oil was chromatographed on 100 g of SiO₂ to give 1.4 g of oil which was a mixture of 4 & 5. The mixture was purified by vacuum distillation, yielding 232 mg of 4. (96.7% pure by gas chromatography).

Hexanoic acid, 4-ethyl-4-phenyl- (6)

The ester (4) was hydrolyzed to the acid (6) by heating in a mixture of ethanol/water (19.8 mL/3.7 mL), sodium hydroxide (4.0 g) for 2 h. The mixture was cooled, diluted with water (222 mL) and acidified with 6 M HCl. The acidified mixture was extracted with CH₂Cl₂ (3 x 50 mL). The combined

extract were washed with water (2 x 75 mL) and concentrated to an oil. The oil was mixed with water (15 mL) and basified with 6 M NaOH. The resulting aqueous suspension was washed with CH₂Cl₂ (3 x 5 mL) then acidified to pH 4 with 6 M HCl. After standing overnight large oil drops had formed at the bottom of the flask. Most of the water was aspirated off and the remainder extracted with CH₂Cl₂ (3 x 5 mL). The combined extract was washed with water (3 x 5 mL), dried (Na₂SO₄) and concentrated to an oil, 0.193 g. The oil was purified by chromatography on a column of silica gel (8.0 g) eluted with hexane:EtOAc (3:1). Fractions containing the product were concentrated to 144 mg of oil. A portion of the material (99 mg) was transmitted to WRAIR on September 16, 1997 (Lot No. NJ23-81-2).

Anal.

| | <u>C</u> | <u>H</u> |
|---|----------|----------|
| Calc'd for C ₁₄ H ₂₀ O ₂ | 75.33 | 9.15 |
| Found | 76.78 | 9.22 |

Spectral Data

Infrared (Neat)

Major bands: ν 3020, 2950, 2650, 1705, 1595, 1495, 1465, 1415, 1380, 1300, 1220, 1155, 1025, 750, 690 cm⁻¹.

Nuclear Magnetic Resonance (CDCl₃)

δ 12.00-8.00 (broad s, 1, CO₂H); 7.32-7.13 (m, 5, aromatic H's); 2.03 (d of t, 2, J= 2.0 and 17.7 Hz, CH₂CO₂H); 2.01 (d of t, 2, J= 2.0 and 17.7 Hz, CH₂); 1.70 (q, 2, J= 7.4 Hz CH₂CH₃); 1.69 (q, 2, J= 7.4 Hz, CH₂CH₃); 0.70 (t, 6, J= 7.4 Hz, 2 x CH₃).

Source of Materials

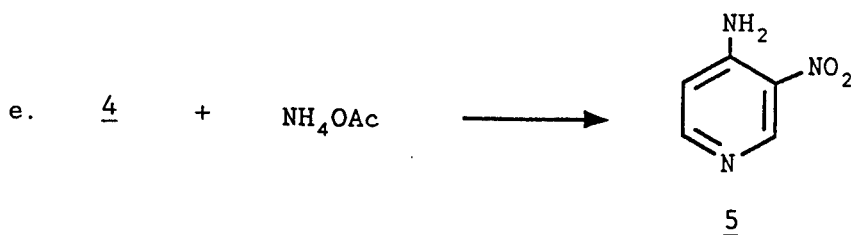
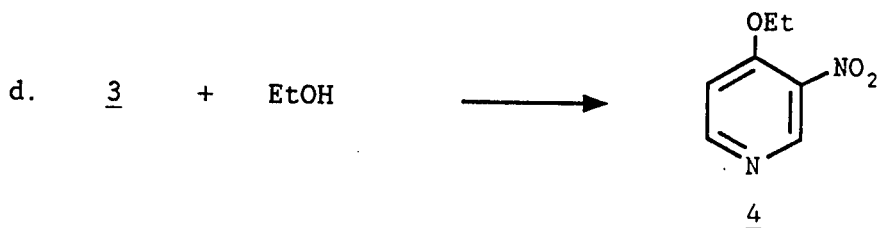
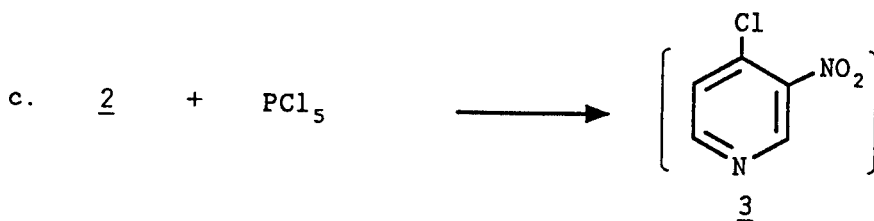
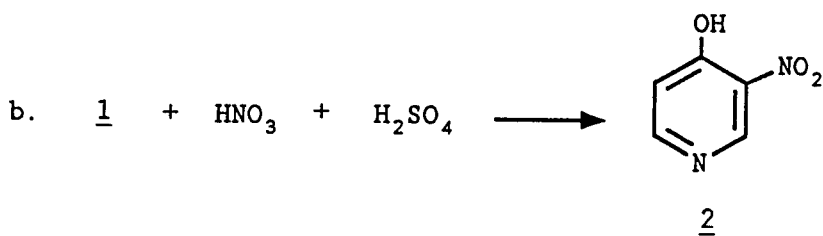
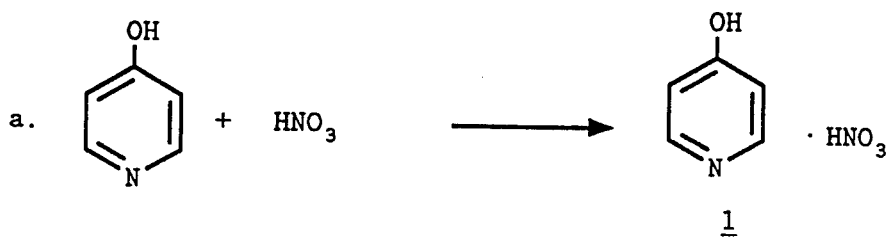
| | | |
|-----|--|----------------------------|
| 1. | 3-Benzoylpropionic acid | Aldrich Chemical Co., Inc. |
| 2. | EtOH | U.S. Industries |
| 3. | H ₂ SO ₄ | J.T. Baker Chemical Co. |
| 4. | CH ₂ Cl ₂ | J.T. Baker Chemical Co. |
| 5. | NaHCO ₃ | Aldrich Chemical Co., Inc. |
| 6. | Ethyl 3-benzoylpropionic acid | Starks Associates, Inc. |
| 7. | Triethylaluminum | Aldrich Chemical Co., Inc. |
| 8. | Triethylsilyl trifluoromethane- sulfonate | Aldrich Chemical Co., Inc. |
| 9. | H ₃ PO ₄ | Aldrich Chemical Co., Inc. |
| 10. | Ether | J.T. Baker Chemical Co. |
| 11. | MeOH | J.T. Baker Chemical Co. |
| 12. | NaOH | Aldrich Chemical Co., Inc. |
| 13. | HCl | J.T. Baker Chemical Co. |

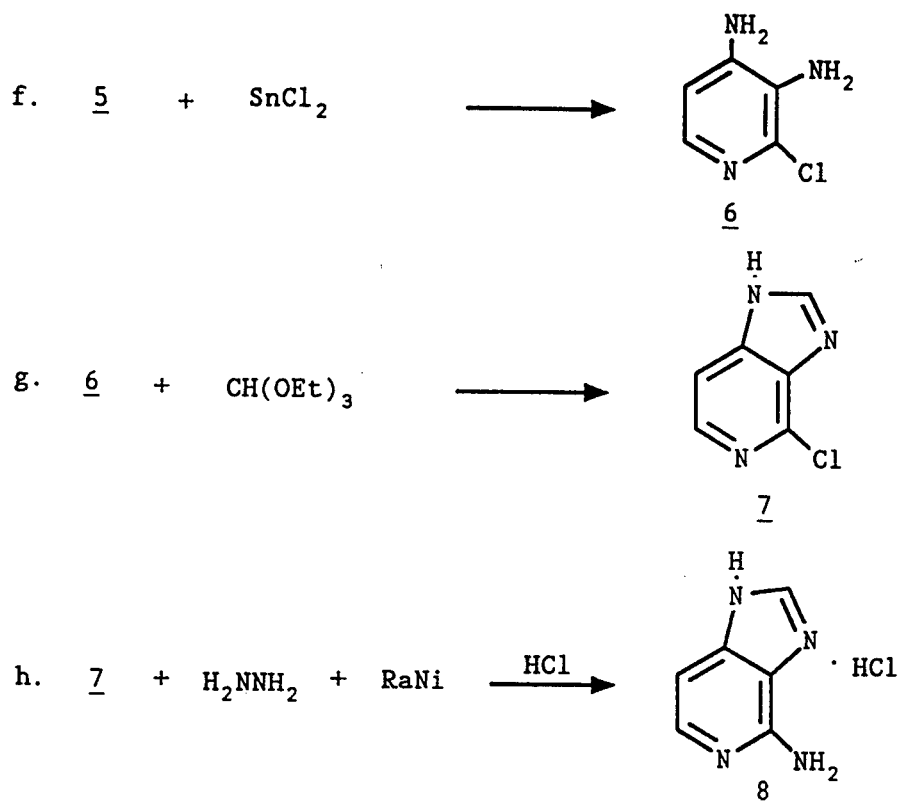
VII. TARGET COMPOUNDS AND INTERMEDIATES IN PROGRESS FROM
DECEMBER 1, 1996 TO NOVEMBER 30, 1997

1. 3. Deazaadenine

The target compound 8 is being prepared by the following sequence of reactions.

Reaction Sequence:





Experimental

Pyridine, 4-hydroxy- nitrate (1)

To a stirred suspension of 4-hydroxypyridine (400.8 g, 4.0 mol) in CH_2Cl_2 (6 L) was added, dropwise, fuming HNO_3 (90%, 280.4 g, 192 mL, 4.0 mol) during 1.5 h. After the addition the suspension was stirred at RT for 16 h. The solid was collected on a filter, washed with CH_2Cl_2 (3 x 1.6 L) then dried; yield, 646.8 g (100%). The material was suitable for further transformation. Additional product (159.4 g) was obtained from a scouting run for a total of 806.2 g (100% overall).

Pyridine, 4-hydroxy-3-nitro- (2)

To stirred 4-hydroxypyridine nitrate (1) (323.4 g, 2.04 mol) was added, dropwise, a mixture of fuming HNO₃ (90%, 425 mL, 631.6 g, 10.02 mol) and fuming H₂SO₄ (25%, 341 mL, 654.7 g, 654.7 g, 6.67 mol) during 0.5 h. After the addition the mixture was slowly heated to 70°C. Exotherm set in, and the temperature of the mixture rose to 90°C. The mixture was heated at 93°C for 1 h, cooled, then poured over 1200 g of ice. The solid that separated was collected, washed with H₂O, then dried; yield 285.8 g. Additional product was obtained from an identical reaction and from a scouting run for a total of 713.1 g (100% overall). The material was recrystallized from H₂O (8.2 l) to give 496.8 g, (71%) of pure 2.

Spectral Data

Nuclear Magnetic Resonance (TFA-d)

δ 11.50 (s, 1H, OH); 9.46 (s, 1H, H at C-2); 8.65 (d, 1H, J= 7.0 Hz, H at C-6); 7.61 (d, 1H, J= 6.9 Hz, H at C-5).

Pyridine, 4-chloro-3-nitro- (3)

A stirred mixture of PCl₅ (696.5 g, 3.34 mol), 4-hydroxy-3-nitropyridine (398.6 g, 2.84 mol), and dichloroethane (2.6 L) was heated under reflux for 5 h. The solution was cooled to 60°C then used immediately without further characterization.

Spectral Data

Infrared (KBr pellet)

ν 3100-2850 (complex pattern); 1595, 1550, 1520, 1460, 1390, 1350, 1310, 1280, 1235, 1195, 1020, 840, 810, 750, 680 cm^{-1} .

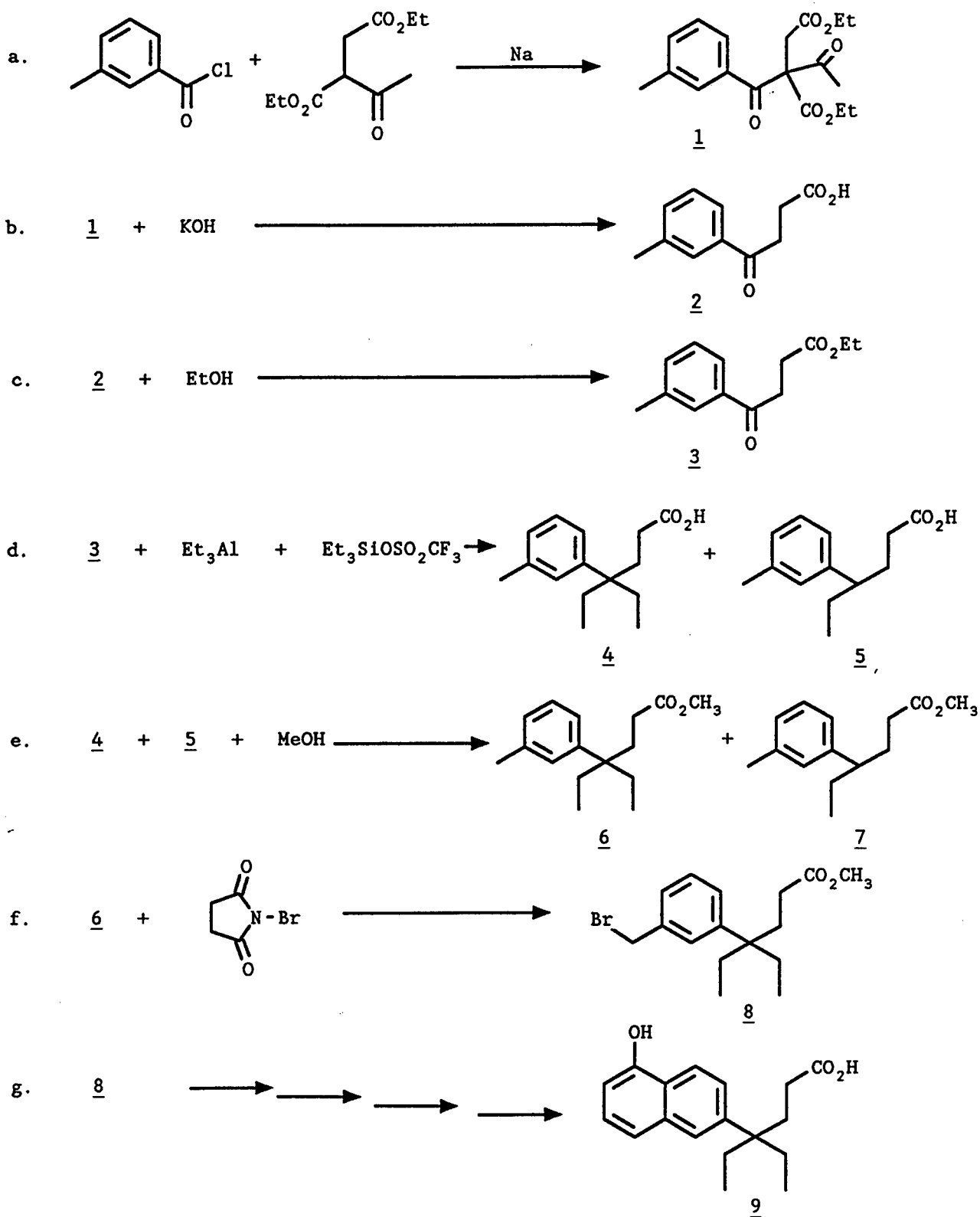
Nuclear Magnetic Resonance (CDCl_3)

δ 9.00 (s, 1H, H at C-2); 8.65 (d, 1H, $J = 6$ Hz, 'H at C-6); 7.75 (d, 1H, $J = 6$ Hz, H at C-5); 4.35 (q, 2H, $J = 7$ Hz, $-\text{CH}_2-$); 1.50 (t, 3H, $J = 7$ Hz, $-\text{CH}_3$).

2. Ligand 09E

The target compound 9 is being prepared by the following sequence of reactions:

Reaction Sequence:



Experimental

Succinic acid, 2-acetyl-2-(3-methylbenzoyl)-, diethyl ester (1)

Hexane washed sodium spheres (10.5 g, 0.457 mol) were suspended in ether (100 mL) under argon and stirred 40 min. A solution of acetyl diethyl succinate (103.9 g, 0.481 mol) in ether (100 mL) was added slowly dropwise over 4 h while keeping the reaction mixture at 25-30°C. After stirring at room temperature overnight, all the sodium had disappeared. The solution was cooled in an ice bath to 3°C and a solution of *m*-tolyl chloride (67.4 g, 0.436 mol) in ether (100 mL) was added dropwise over 1.5 h. The mixture was stirred in the cold for 4.5 h following the addition. The ice bath was removed and the mixture stirred overnight while warming to room temperature. The reaction mixture was mixed with water (500 mL) and the layers separated. The aqueous portion was extracted with ether (2 x 250 mL) and the combined ethereal portions were washed with saturated aqueous sodium bicarbonate (2 x 250 mL). The washed solution was dried over Na₂SO₄ and concentrated to 153.8 g of yellow oil. The oil was dissolved in 200 mL hexane:EtOAc (7:3) and purified on a 3.4 kg column of silica gel eluted with hexanes:EtOAc (7:3). Fractions containing product 1 were concentrated to 135.6 g of oil (0.406 mol, 93.0%).

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.58 (s, 1, H at C-2); 7.49 (d, 1, J= 7.8 Hz, H at C-6); 7.35 (d, 1, J= 7.5 Hz, H at C-4); 7.29 (m, 1, H at C-5); 4.27 (q, 2, J= 7.1 Hz, COOCH₂); 4.12 (q, 2, J= 7.2 Hz, COOCH₂); 3.23 (s, 2, CH₂CO); 2.43 (s, 3, COCH₃); 2.38 (s, 3, CH₃); 1.22 (m, 6, 2 x CH₃).

Propionic acid, 3-(3-methylbenzoyl)- (2)

The ester (1) (135.6 g, 0.406 mol) was stirred vigorously at room temperature in 4 L of 0.25 M aqueous potassium hydroxide for several hours so that the oil was well dispersed. The mixture was then stirred gently over the weekend. After stirring for about 70 h, some oil remained at the bottom of the flask. The aqueous portion was decanted and the oil mixed with ether (50 mL) and water (50 mL). After separation, the aqueous portion was added to the decanted portion and the solvent was removed from the organic portion to yield 33.6 g of oil which was set aside for further hydrolysis. The aqueous portion was acidified with conc. HCl (80-90 mL) and extracted with ether (4 x 500 mL). The combined extract was washed with water (300 mL), dried over MgSO_4 and concentrated to 46.2 g of oil which crystallized to an off-white solid. The solid was recrystallized from hexanes:EtOAc (7:3) to yield 19.1 g pure compound 2, mp 109-114°C. The mother liquor was set aside for later purification. The oil which had been recovered (30.1 g, 90 mmol) was hydrolyzed by dissolving in ethanol (480 mL), adding 85% KOH (14.7 g, 0.223 mol) and water (320 mL) and stirring at room temperature overnight. The mixture was acidified by adding conc. HCl (20-22 mL), then concentrated to remove the ethanol. The remaining aqueous portion (~150 mL) was extracted with dichloromethane (3 x 150 mL). The combined extract was washed with water (2 x 50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated to 17.9 g of a solid. The solid was recrystallized from hexanes:EtOAc (8:3) to yield 4.5 g of pure compound 2, mp 114-115°C. The mother liquor was combined with the mother liquor from the previous recrystallization and purified on a 2.29 kg column of silica gel eluted with hexanes:EtOAc (7:3). Fractions containing the product were combined and concentrated to 7.5 g of impure solid. The solid was recrystallized from hexanes:EtOAc (8:3) to yield 3.9 g of pure compound 2, mp 116-118°C. Total compound 2 prepared, 27.5 g (0.143 mol, 35.3%).

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 10.50 (br, 1, COOH); 7.79-7.77 (m, 2, aromatic H's); 7.39-7.54 (m, 2, aromatic H's); 3.31 (t, 2, J= 6.6 Hz, COCH₂); 2.81 (t, 2, J= 6.6 Hz, CH₂CO₂H), 2.41 (s, 3, CH₃).

Propionic acid, 3-(3-methylbenzoyl)- ethyl ester (3)

Dissolved compound 2 (19.1, 99.4 mmol) in ethanol (35 mL) and added 0.71 g conc. sulfuric acid. The stirred mixture was heated at reflux temperature (78°C) for 6 h, checking the progress of the reaction by TLC at two hour intervals. After cooling and standing overnight, the mixture was concentrated to an oil. The oil was dissolved in dichloromethane (175 mL) and washed with saturated aqueous sodium bicarbonate (2 x 90 mL) and water (90 mL). The solution was dried (MgSO₄) and concentrated to 20.3 g oil (92.2 mmol, 92.7%) judged to be over 95% pure on the basis of TLC and high field NMR.

Spectral Data

Nuclear Magnetic Resonance (CDCl₃)

δ 7.79 (m, 2, aromatic H's); 7.37 (m, 2, aromatic H's); 4.17 (q, 2, J= 7.0 Hz CH₂CH₃); 3.31 (t, 2, J= 6.7 Hz, COCH₂); 2.76 (t, 2, J= 6.4 Hz, CH₂CO₂H), 2.41 (s, 3, CH₃); 1.27 (t, 3, J= 6.7, CH₂CH₃).

Butyric acid, a mixture of 4,4-diethyl-4-(3-methylbenzoyl)-
(4) and 4-ethyl-4-(3-methylbenzoyl)- (5)

Dissolved compound 3 (11.0 g, 49.9 mmol) in dichloromethane (80 mL) under argon and cooled to 3°C in an ice bath. A 1 M solution of triethyl aluminum in hexanes (100 mL, 100 mmol) was added dropwise while keeping the reaction mixture at 3-5°C. The mixture was stirred 20 min., then triethyltrifluoromethylsulfonate (26.4 g, 100 mmol) was added dropwise still keeping the reaction mixture at 3-5°C. The mixture was stirred for 1 h, then the ice bath was removed and the mixture was kept at room temperature over the weekend. The reaction was quenched by carefully pouring it into a stirred mixture of ice, water (250 mL) and ether (225 mL). Approximately 2 mL of phosphoric acid was added and, after mixing, the aqueous and organic phases were separated. The aqueous portion was extracted with ether (3 x 275 mL), then the combined extract was washed with brine (2 x 275 mL), dried (MgSO₄), and concentrated to 19.1 g of oil. The oil was dissolved in 20 mL hexanes:EtOAc (7:3) and partially purified on a column of silica gel (375 g) eluted with hexanes:EtOAc (7:3). Fractions containing the product (4 and 5) were concentrated to 12.7 g of oil (109%).

Butyric acid, a mixture of ethyl esters of 4,4-diethyl-4-
(3-methylbenzoyl)- (6) and 4-ethyl-(3-methylbenzoyl)- (7)

To facilitate purification by vacuum distillation, the acids 4 and 5 (12.7 g) were esterified by refluxing in methanol (25 mL) with concentrated sulfuric acid (0.50 g) for 2.25 h. After cooling, the solvent was removed and the residue mixed with dichloromethane (100 mL) then washed with saturated aqueous sodium bicarbonate (50 mL) and water (2 x 50 mL). The solution was dried over MgSO₄ and concentrated to 11.5 g of oil. Analysis by gas chromatography showed that the

oil contained 23.5% of compound 6 and 32.3% of compound 7. The oil was vacuum distilled using an oscillating Kugelrohr apparatus and a slow ramp of temperature from 66°C to 74°C (pressure ranged from 0.05 mm to 0.015 mm). Gas chromatography was used to monitor the composition of the fractions. When the distillation was completed, the pot residue consisted of a mixture enriched in compound 6 (67.7%) and containing only a small amount (5.0%) of compound 7; total 2.00 g. Some of the later fractions which contained a significant portion of compound 6 were combined for redistillation. Upon completion of this second distillation, 0.65 g of oil remained in the pot and had a composition similar to the first, i.e. 67.9% of compound 6 and 7.9% of compound 7. (By contrast, the collected distillate (1.976 g) contained 71.1% of compound 7 and 19.1% of compound 6). To further purify the product (6) the pot residues (2.00 g + 0.65 g) were combined and hydrolyzed back to the acid (4 and 5) by refluxing in a mixture of ethanol (114 mL), water (21.3 mL) and sodium hydroxide (23.0 g, 575 mmol) for 2 h. TLC (hexanes:EtOAc, 7:3) showed no starting ester remaining. After cooling briefly, the mixture was poured into water (1280 mL) and acidified with 6 M HCl (95 mL). The mixture was extracted with dichloromethane (4 x 300 mL) and the combined extract concentrated to 3.1 g of oil. The oil was suspended in 85 mL water and brought to pH ~10 with 6 M NaOH. The mixture was washed with dichloromethane (3 x 30 mL) then re-acidified to pH ~3 with 6 M HCl resulting in the precipitation of an oil. After standing several min, the supernatant water was decanted and extracted with dichloromethane (2 x 30 mL). The oil, dissolved in 30 mL dichloromethane, was combined with the extracts, washed with water (3 x 30 mL), dried (Na₂SO₄) and concentrated to 2.09 g oil. The oil was then dissolved in 4 mL hexanes:EtOAc (9:1) and purified on a 120 g column of silica gel packed in hexanes:EtOAc (9:1) and eluted with hexanes:EtOAc (7:3). Fractions containing the product 4 (and, presumably 5) were isolated as two lots; (a) 1.17 g, pure by

TLC and showing minor impurity peaks on high field NMR and (b) 0.48 g, one impurity spot on TLC and significant impurity peaks on high field NMR. Lot (a) (1.11 g) was re-esterified by refluxing for 2 h in a mixture of methanol (2.5 mL) and conc. H_2SO_4 (50 mg - two drops). After removal of solvent, the oily residue was dissolved in dichloromethane (8.8 mL), washed with saturated aqueous sodium bicarbonate (4.4 mL) and water (2 x 4.4 mL). The solution was dried (MgSO_4) and concentrated to 1.17 g oil (4.71 mmol, 99.5%). TLC in hexanes:EtOAc (7:3) gave a single spot ($R_f=0.75$) and GC analysis showed 91.9% compound 6 and 5.1% compound 7. High field NMR agreed with the structure of 6 and showed only minor impurity peaks. Lot (b) (.43 g) was re-esterified as described above to yield 0.416 g of compound 6 (plus compound 7 and other impurities) = 1.68 mmol (~91%); high field NMR agrees with the structure of 6 but shows significant impurity peaks; TLC (hexanes:EtOAc 7:3) shows three minor impurity spots in addition to the major spot representing compound 6. This lot was judged suitable for a bromination scouting run and subsequent purification.

Butyric acid, 4,4-diethyl-4-(3-bromomethylphenyl)-methyl ester (8)

A stirred mixture of 6 (0.406 g, 1.63 mmol), N-bromo-succinimide (0.350 g, 1.97 mmol) and benzoyl peroxide (4 mg, 0.02 mmol) in carbon tetrachloride (6 mL) was heated at reflux (78°C) for 4.5 h. After standing at room temperature overnight, the mixture was filtered to remove succinimide and the filter cake washed with hexanes:EtOAc (7:3). The filtrate was concentrated to an oil, then re-dissolved in ~1 mL hexane:EtOAc (9:1) and purified on a 45 g column of silica gel packed in hexane:EtOAc (9:1) and eluted with hexanes:EtOAc (7:3). Fractions containing the product (8) were combined and concentrated to 0.327 g oil (0.999 mmol, 61.1%). TLC shows

a single major spot ($R_f = 0.61$) with faint impurity spots just above and below the product spot. High field NMR agrees with the structure of **8** and shows only a few minor impurity peaks.

Spectral Data

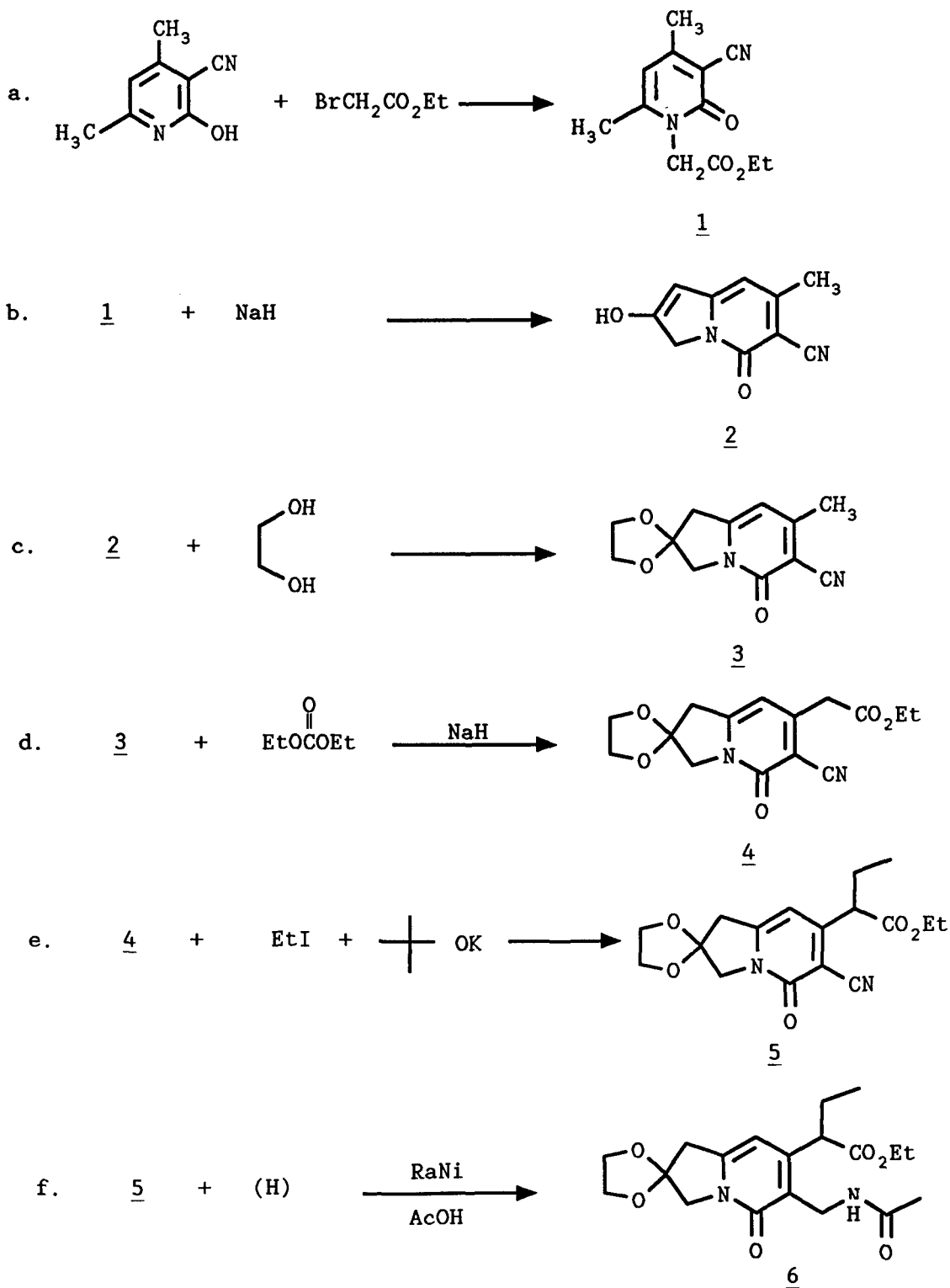
Nuclear Magnetic Resonance (CDCl_3)

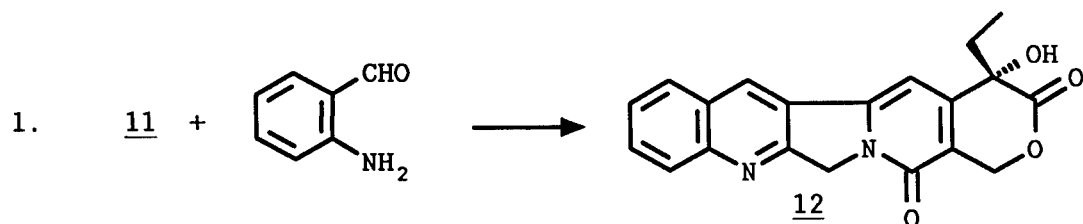
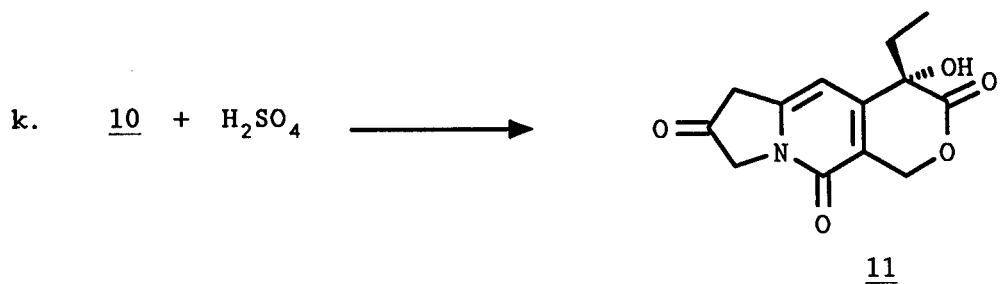
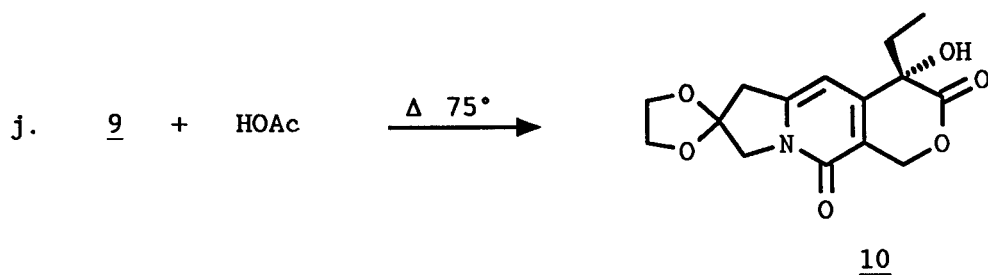
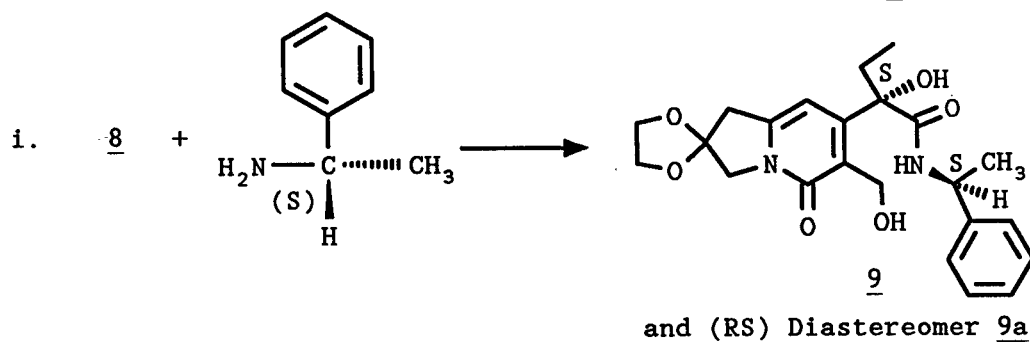
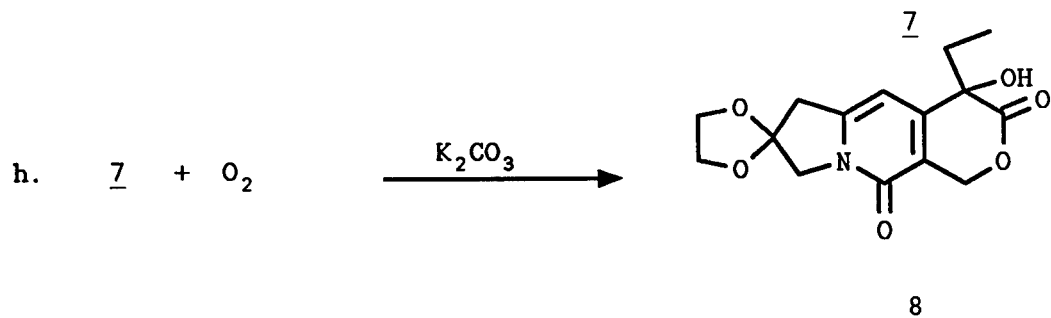
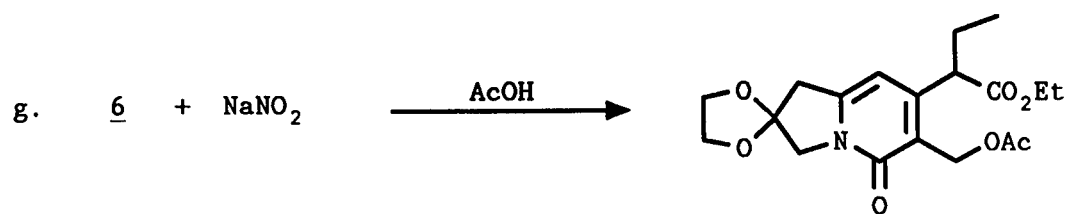
δ 7.46-7.21 (m, 4, aromatic H's); 4.50 (s, 2, CH_2Br); 3.61 (s, 3, CO_2CH_3); 2.02 (m, 4, $\text{CH}_2 \times 2$); 1.70 (m, 4, 2 \times CH_2); 0.69 (m, 6, 2 \times CH_3).

3. (S)-7-Ethyl-7-hydroxy-10(H)-pyrano[3',4':6,7]indolizino-[2,1-b]quinoline-8,11(7H,13H)-dione (12)

The target compound 12 is being prepared by the following sequence of reactions.

Reaction Sequence:





Experimental

Acetic acid, 2-[3-cyano-4,6-dimethyl-2-pyridone-1-yl]-ethyl ester (1)

To a solution of 3-cyano-4,6-dimethyl-2-hydroxypyridine (10.0 g, 6.75 mmol) and KOH (4.4 g of 85%, 6.7 mmol) in 2-methoxyethanol (100 mL) was added ethyl bromoacetate (12.0 g, 7.18 mmol), and the mixture was heated under reflux for an hour then cooled. The solid was filtered off, washed with 2-methoxyethanol, then discarded. To the filtrate was added conc. NH_4OH (1.6 mL) and H_2O (375 mL). The mixture was stirred for 20 min then filtered. The collected solid (9.8 g, 62.0%) was suitable for further transformation. Additional product (4.0 g) was obtained from a scouting run.

Indolizine, 6-cyano-2,5-dihydroxy-7-methyl- (2)

Sodium hydride (0.7 g of 60%, 17.5 mmol) was added to THF (250 mL), and the suspension was cooled in an ice bath. To this was added 1 (4.0 g, 17.1 mmol) and the mixture was stirred at RT, under an argon atmosphere, for 20 h then poured into H_2O (400 mL) and acidified with HCl. The solid that separated was collected on a filter, washed with H_2O (2 x 50 mL), then crystallized from EtOH (400 mL); yield 2.5 g (77.9%). The material was suitable for further transformation. Additional product (5.1 g) was obtained from a larger run.

6-Cyano-2,2-(ethylenedioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydroindolizine (3)

A mixture of 2 (2.0 g, 10.63 mmol), ethylene glycol (36 mL) and *p*-toluenesulfonic acid (169 mg, 0.89 mmol) in toluene

(300 mL) was refluxed using Dean Stark trap, for 5 h. The toluene layer was decanted and fresh toluene (200 mL) was added. The reaction mixture was refluxed for an additional 5 h and the toluene layer decanted as before. The toluene layers were combined, washed with brine (3 x 50 mL), dried over Na_2SO_4 , and evaporated to yield the crude product. The material was purified by chromatography on 100 g SiO_2 using $\text{EtOAc}/\text{CH}_2\text{Cl}_2$ (1:1) as the eluent to obtain 450 mg (15.5%) of 3, mp 180-181°C.

Anal.

| | <u>C</u> | <u>H</u> | <u>N</u> |
|---|----------|----------|----------|
| Calc'd for $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_3$ | 62.06 | 5.21 | 12.06 |
| Found | 62.20 | 5.39 | 11.74 |
| | 62.13 | 5.43 | 11.65 |

Spectral Data

Infrared (KBr)

Major bands: 3079, 2967, 2933, 2895, 2213, 1650, 1596, 1538, 1434, 1342, 1277, 1230, 1211, 1138, 1040, 1006, 946, 915, 854, 802, 771, 738, 651, 612, 508 cm^{-1} .

Nuclear Magnetic Resonance (CDCl_3)

δ 6.10 (s, 1, H at C-8); 4.13 (s, 2, NCH_2); 4.04 (m, 4, $\text{OCH}_2\text{CH}_2\text{O}$); 3.32 (d, 2, H at C-1); 2.4 (s, 3, CH_3).

Mass Spectrometry (Electron impact)

m/e [Rel. int., ID]: 234 [5, $(m+2)^+$]; 233 [36, $(m+1)^+$]; 232 [100, m^+]; 160 [75, $(m - \text{C}_3\text{H}_4\text{O}_2)^+$].

Thin Layer Chromatography

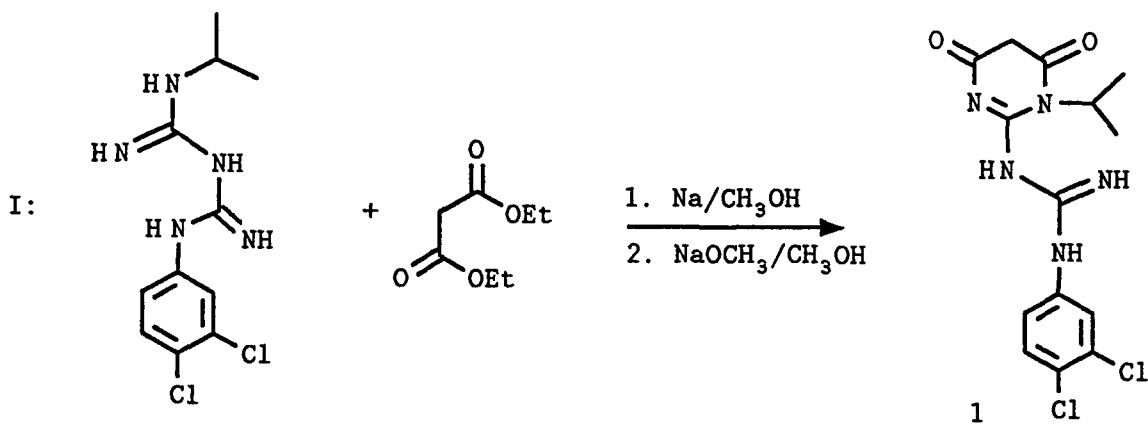
EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

| | <u>Eluent</u> | <u>Rf Value</u> | <u>Comment</u> |
|----|---|-----------------|----------------|
| 1. | EtOAc-CH ₂ Cl ₂ (1:1) | 0.43 | single spot |

VIII. ATTEMPTED SYNTHESSES

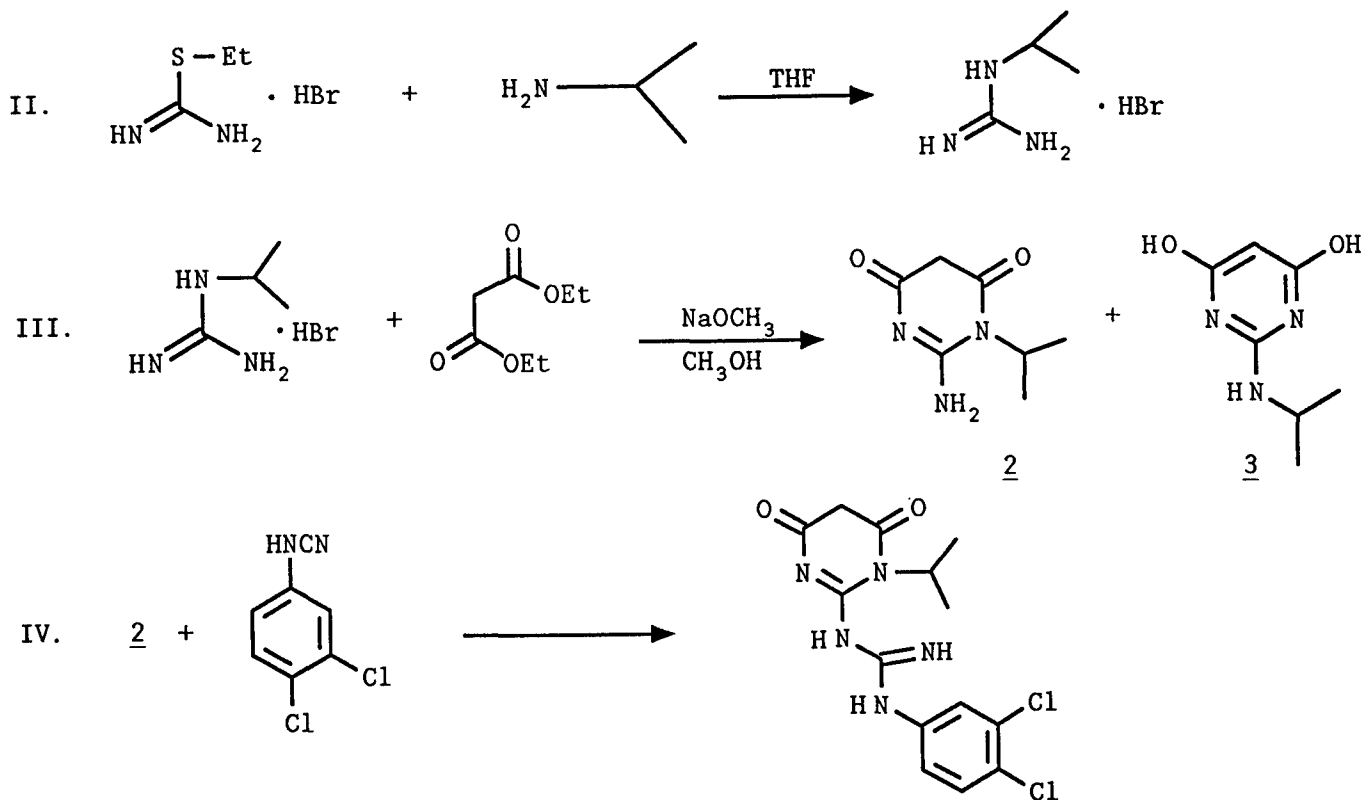
1. 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,6-dioxo-2-pyrimidinylidene)guanidine

Reaction Sequence:



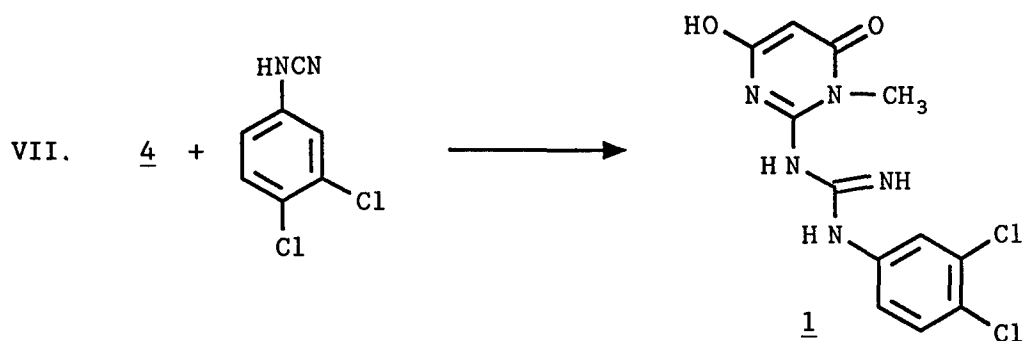
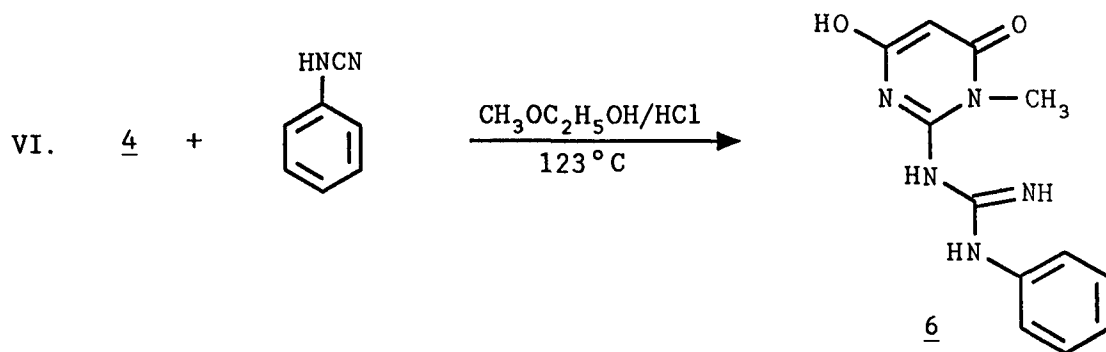
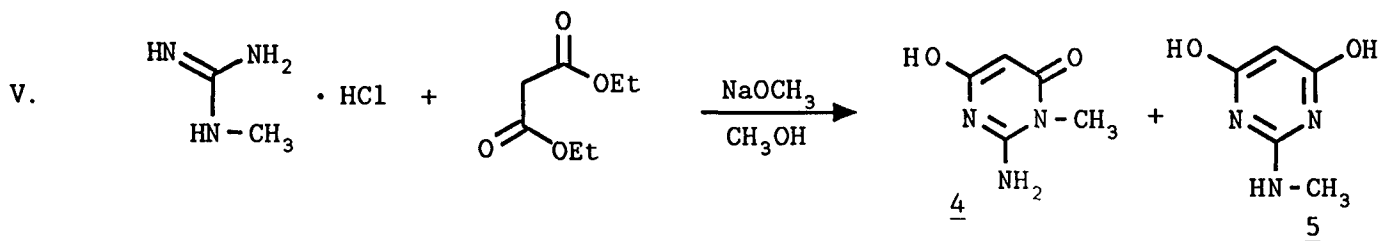
The above reaction was run twice as shown; first by forming sodium methoxide from sodium and methanol and second, by using commercially prepared sodium methoxide (25% in methanol). Although the NMR spectrum was encouraging, mass spectra revealed a molecular wt. of 369 instead of the expected 355. Two further reactions were run; one using malonyl dichloride in dichloromethane with pyridine and the other using diethyl succinate in methanol/sodium methoxide. In all cases, the desired product 1 was not obtained and alternative methods were sought.

The reaction scheme shown below was proposed.



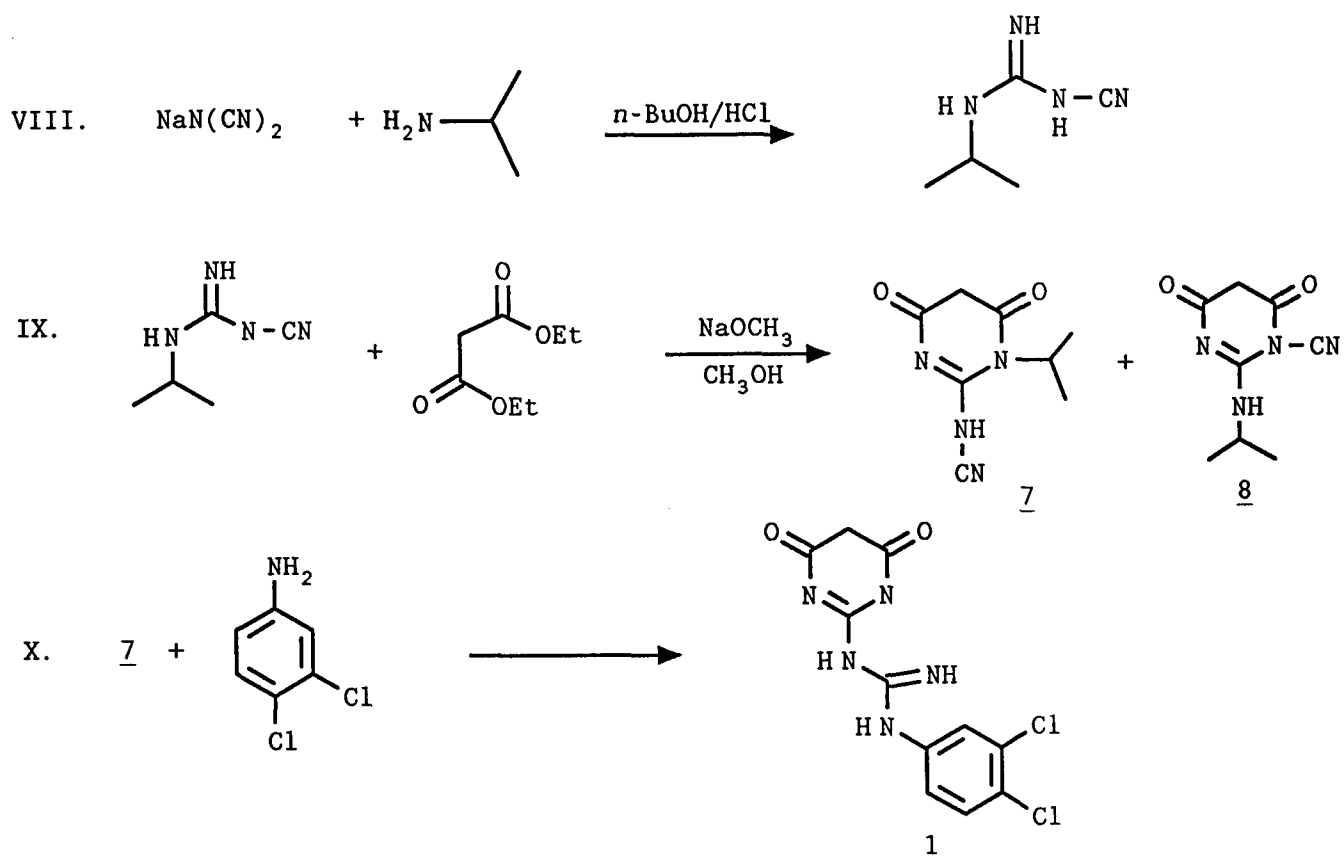
The starting material, isopropyl guanidine, was prepared as shown above, (Rxn II) and confirmed by NMR. It was then reacted in the second step with diethyl malonate, but the only product isolated was compound 3 as confirmed by its NMR spectrum. Because of this, the third and final step was not attempted. Steric hindrance due to the isopropyl group was suspected of inhibiting the formation of compound 2.

A model reaction sequence using a methyl group (instead of an isopropyl group) was attempted in order to investigate this approach further.



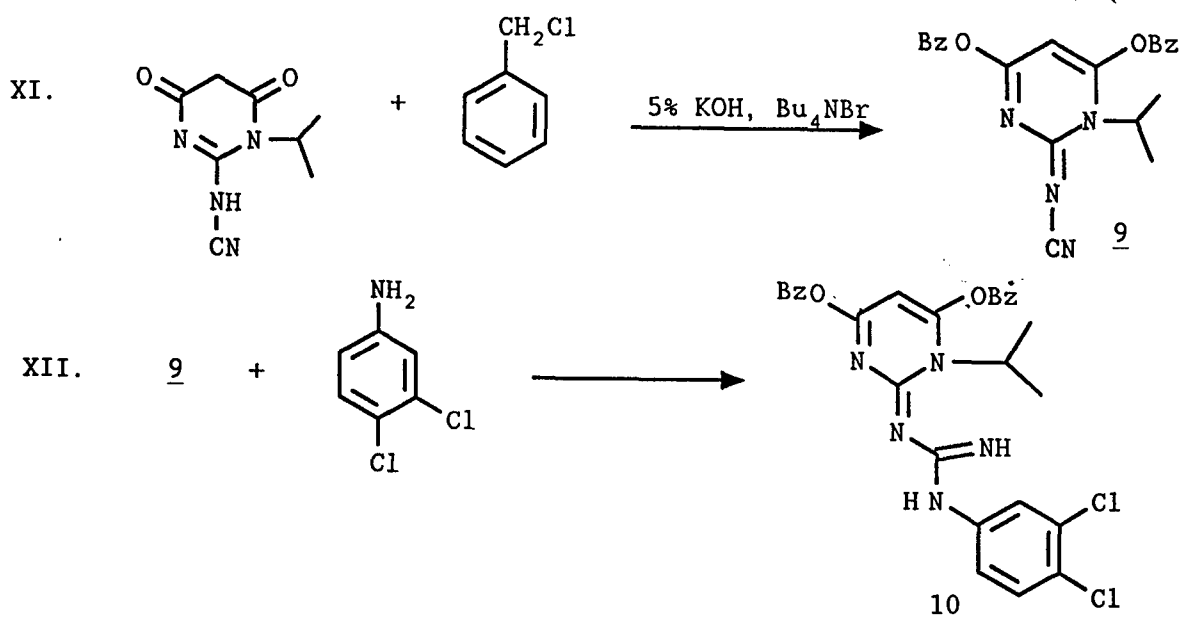
The reaction V was carried out successfully giving an overall yield of 65% (both products); compound 4 predominated giving 73% of the total. The two products can be distinguished from each other by the differences in their NMR spectra. By repeated recrystallization, 4 and 5 were separated yielding sufficient 4 for the next step. Phenylcyanamide was made by reacting aniline and cyanogen bromide; it was used as soon as possible since it was unstable, converting to a high melting solid (probably a dimer) within 24 h. The reaction VI between 4 and phenylcyanamide yielded

several products, none of which was the desired compound 6. Since it was suspected that the instability of phenylcyanamide contributed to the failure of reaction VI, the much more stable dichlorophenylcyanamide was prepared. The reaction VII, however, was never carried out. Because of the difficulties encountered in the previous reactions, the following reaction sequence was investigated.



A few large runs of reaction VIII sufficed to prepare enough isopropylidicyandiamide for a dozen or so runs of reaction IX. Compound 7 of reaction IX was typically purified by chromatography on silica gel followed by precipitation from aqueous solution by adding acid. Reaction X was tried over a dozen times varying time (1-23 hrs.), temperature (5-135°C) and solvent (ethanol, methanol, methoxyethanol and ethoxyethanol) with acid (conc. HCl, anhydrous HCl) or base (KOH), or neither. Following the reaction, the solvent was removed in vacuo and a solid was obtained either directly or

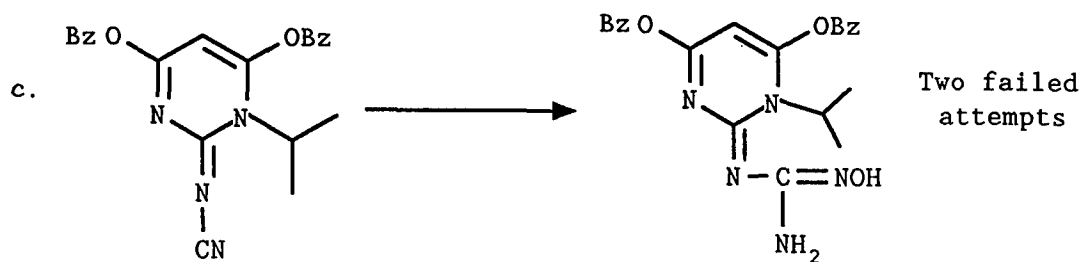
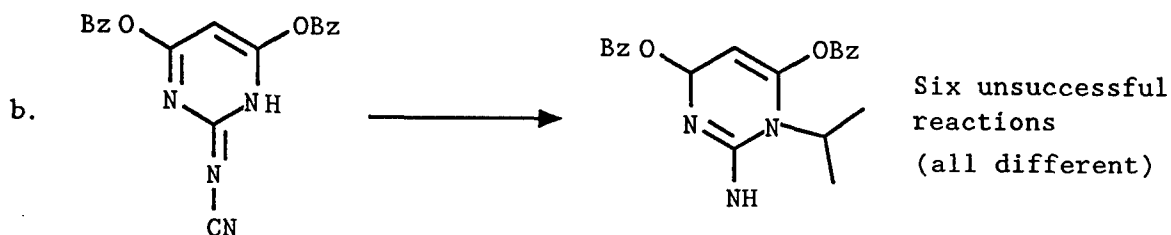
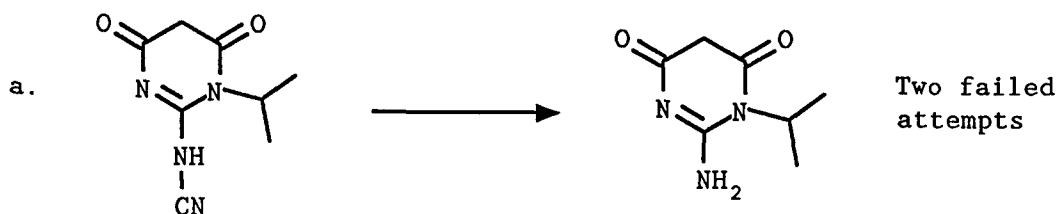
following trituration in EtOAc. The solid was then subjected to chromatography on silica gel and the more promising components investigated most commonly, by thin layer chromatography and NMR spectra. In all cases, no evidence of compound 1 was found. Since it was considered possible that the oxygen atom on the ring might react, the reaction scheme below (XI, XII) was carried out.



The reaction XI was carried out successfully several times. After stirring the reactants at room temperature (typically 2-3 days) the methylene chloride layer was separated, washed, dried and the solvent removed. The product (compound 9) was purified by chromatography on silica gel. Alternately compound 9 was also prepared by stirring at room temperature a mixture of 7, benzyl chloride and silver oxide in DMF for 1-3 days. After filtration of the reaction mixture and concentration of the filtrate, the material was ready for chromatographic purification. Reaction XII was run over a dozen times varying time (1 - 23 hrs), temperature (40-200°C), and solvent (five different solvents, and one reaction run neat at 150°C). In addition, the use of various acid or base facilitators were explored, including conc. HCl, dry HCl gas,

AlCl_3 , ZnCl_2 , KOH , LiOH and $(\text{CH}_3)_3\text{COK}$. Following the reaction, the mixture was checked by TLC to confirm that a reaction had taken place (about one third showed no reaction). Those mixtures which showed evidence of reaction were worked up and purified as previously done for reaction X. However, no evidence of compound 10 was found.

Topics not included in the summary.



2. 7-Chloro-3-(2,4-dichlorophenyl)-1-(4-dimethylamino-1-butenyl)-3,4-dihydro-9(10H)-acridinone (1)

Attempts to prepare target compound (1) are summarized in table 1. Initial attempts reacting diketone (2) with Wittig ylid (8a) gave either no reaction (examples 1 and 2) or no new isolatable products (example 3). Diketone (2) was then converted to mono-ketone (3) and the Wittig reactions were tried (examples 5 and 6), however, again no new isolatable products were formed. At this time, mono-ketone (4) was prepared and a variety of Wittig reactions were run (examples 7-22). In all cases, either no reaction occurred, no desired product was formed/isolated, nor no new isolatable products were formed. In light of the lack of success utilizing the tricyclic ketones (2-4), mono- and diketones (5) and (6) were prepared and Wittig reactions were attempted (examples 23-31). Unfortunately, formation of the Wittig product (7a/7b), which could be converted to target compound (1), did not occur. The only reaction which provided a reasonable yield (example 28), in fact gave what appears to be an unexpected product (7c).

Compounds 1 - 9b

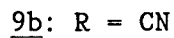
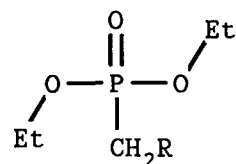
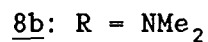
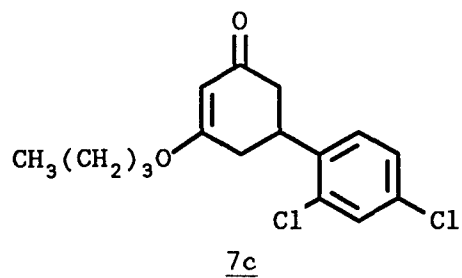
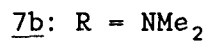
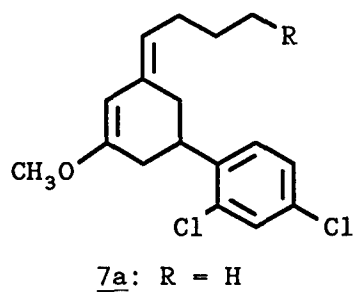
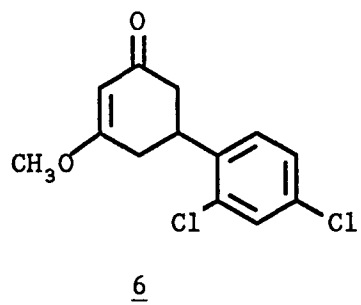
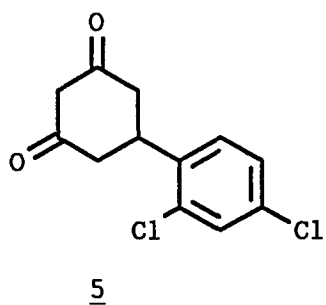
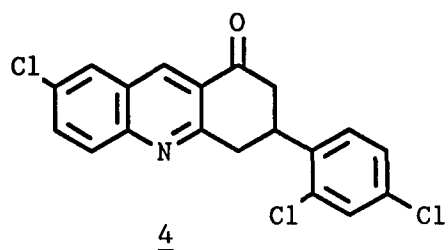
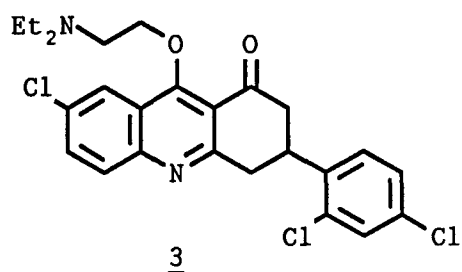
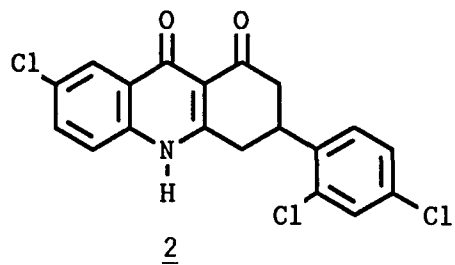
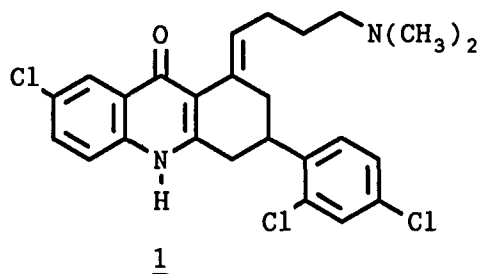


TABLE 1
(Examples 1-31)

| Reactant | "YLID" (equiv. used) | Base ¹ | Solvent ² | Temp. | Time | Results ³ |
|----------|-------------------------|-------------------|----------------------|------------------|-------|----------------------|
| 2 | 8a (1) | b | a | RT | 3 h | a |
| 2 | 8a (2) | b | a | RT | 70 h | a |
| 2 | 8a (2) | b | a | reflux | 18 h | b |
| 2 | 9a (1) | c | d | RT | 18 h | a |
| 3 | 8b (1.2) | a | a | -30→RT | 18 h | b |
| 3 | 8b (2.3) | a | a | -30→RT | 100 h | b |
| 4 | 8b (1) | a | a | RT | 24 h | a |
| 4 | 8b (2.6) | a | a | RT | 24 h | a |
| 4 | 8a (3) | b | a | RT→reflux →RT | 70 h | c |
| 4 | 8a (4) | b | a | RT | 70 h | c |
| 4 | 8a (1) | b | a | reflux | 6 h | a |
| 4 | 8a (1.2) | b | a | reflux | 6 h | c |
| 4 | 8a (2) | b | a | reflux | 6 h | c |
| 4 | 8a (1.4) | b | b | reflux | 6 h | a |
| 4 | 8a (1.2) | b | b | reflux | 16 h | a |
| 4 | 8a (2) | b | b | reflux | 16 h | b |
| 4 | 8a (4) | b | b | reflux | 16 h | b |
| 4 | 8b (1) | c | c | RT→reflux | 16 h | a |
| 4 | 8b (1) | b | a | RT→reflux | 15 h | a |
| 4 | 8b (1) | b | a | -78 → RT | 20 h | b |
| 4 | 9a (1.2) | d | e | RT | 20 h | b |
| 4 | 9a (1.2) | c | d | reflux | 21 h | a |
| 5 | 8b (1) | a | a | RT | 16 h | c |
| 6 | 9a (1.3) | c | a | RT | 15 h | a |
| 6 | 9a (1) | c | d | RT→reflux | 9 h | a |
| 6 | 8a (0.85) | b | a | RT | 2 h | c |
| 6 | 8a (1) | b | a | -78→RT | 18 h | c |
| 6 | 8a (1.3) | b | a | -78→RT→reflux | 16 h | e (65%) |
| 6 | 8a (2) | b | a | -78→RT→reflux | 34 h | e (-) |
| 6 | 9b (1.1) | c | d | RT→reflux | 18 h | d (-) |
| 6 | 9b (1.5) | c | d | RT→reflux | 19 h | d (5%) |

-
- Base used to generate ylid: a= (TMS)₂NK, b= nBuLi, C= NaH, d= DBU
 - Solvent used: a= THF, b= Et₂O, c= DMF, d= DME, e= CH₃CN
 - Results classified as: a= no reaction, b= no isolated products, c= no desired product, d= desired product, isolated (yield), e= unexpected product isolated (yield)

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